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05JUL03 E820227-1 D02973
P01/7700 0.00-0315691.6

Request for grant of a patent

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The Patent Office

Cardiff Road
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1. Your reference — JHC/P41070GB2

2. Patent application number *(The Patent Office will fill in this part)* — 0315691.6

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Trigen Limited
20 St James's Street
LONDON
SW1A 1ES

Patents ADP number *(if you know it)* — 7516081001

If the applicant is a corporate body, give the country/state of its incorporation — England

4. Title of the invention — Boropeptides

5. Name of your agent *(if you have one)*

Harrison Goddard Foote

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Belgrave Hall
Belgrave Street
Leeds
LS2 8DD

Patents ADP number *(if you know it)* — ~~14571001~~ 7631310002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	74
Claim *(s)*	12
Abstract	1
Drawing *(s)*	3

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature

Date 3 July 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

Jonathan Couchman 0113 233 0100

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Notes

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TITLE OF THE INVENTION

BOROPEPTIDES

BACKGROUND OF THE INVENTION

The present invention relates to pharmaceutically useful products obtainable from organoboronic acids. The application also relates to the use of members of the aforesaid class of products, to their formulation and to other subject matter.

Boronic Acid Compounds

It has been known for some years that boronic acid compounds and their derivatives, e.g. esters, have biological activities, notably as inhibitors or substrates of proteases. For example, Koehler et al. *Biochemistry* 10: 2477 (1971) report that 2-phenylethane boronic acid inhibits the serine protease chymotrypsin at millimolar levels. The inhibition of chymotrypsin and subtilisin by arylboronic acids (phenylboronic acid, m-nitro-phenylboronic acid, m-aminophenylboronic acid, m-bromophenylboronic acid) is reported by Phillip et al, *Proc. Nat. Acad. Sci. USA* 68: 478-480 (1971). A study of the inhibition of subtilisin Carlsberg by a variety of boronic acids, especially phenyl boronic acids substituted by Cl, Br, CH_3, H_2N, MeO and others, is described by Seufer-Wasserthal et al, *Biorg. Med. Chem.* 2(1): 35-48 (1994).

In describing inhibitors or substrates of proteases, P1, P2, P3, etc. designate substrate or inhibitor residues which are amino-terminal to the scissile peptide bond, and S1, S2, S3, etc., designate the corresponding subsites of the cognate protease in accordance with: Schechter, I. and Berger, A. On the Size of the Active Site in Proteases, *Biochem.Biophys.Res.Comm.*, **1967**, *27*, 157-162. In thrombin, the S1 binding site or "specificity pocket" is a well defined slit in the enzyme, whilst the S2 and S3 binding subsites (also respectively called the proximal and distal hydrophobic pockets) are hydrophobic and interact strongly with, respectively, Pro and D-Phe, amongst others.

Pharmaceutical research into serine protease inhibitors has moved from the simple arylboronic acids to boropeptides, i.e. peptides containing a boronic acid analogue of an α-amino carboxylic acid. The boronic acid may be derivatised, often to form an ester. Shenvi (EP-A-145441 and US 4499082) disclosed that peptides containing an α-aminoboronic acid with a neutral side chain were effective inhibitors of elastase and has been followed by numerous patent publications relating to boropeptide inhibitors of serine proteases. Specific, tight binding boronic acid inhibitors have been reported for elastase (K_i, 0.25nM), chymotrypsin (K_i, 0.25nM), cathepsin G (K_i, 21nM), α-lytic protease (K_i, 0.25nM), dipeptidyl aminopeptidase type IV (K_i, 16pM) and more recently thrombin (Ac-D-Phe-Pro-boroArg-OH (DuP 714 initial K_i 1.2nM).

Claeson et al (US 5574014 and others) and Kakkar et al (WO 92/07869 and family members including US 5648338) disclose thrombin inhibitors having a neutral C-terminal side chain, for example an alkyl or alkoxyalkyl side chain.

Modifications of the compounds described by Kakkar et al are included in WO 96/25427, directed to peptidyl serine protease inhibitors in which the P2-P1 natural peptide linkage is replaced by another linkage. The aforesaid PCT application and its corresponding US patent (US 6127340) are included herein by reference, in particular the hydrophobic P3 and P2 residues described therein, the non-basic (hydrophobic) P1 residues described therein, and the described non-natural peptide linkages and their synthesis. As examples of non-natural peptide linkages may be mentioned $-CO_2-$, $-CH_2O-$, -NHCO-, $CHYCH_2-$, -CH=CH-, $-CO(CH_2)_pCO-$ where p is 1, 2 or 3, -COCHY-, $-CO_2-CH_2NH-$, -CHY-NX-, $-N(X)CH_2-N(X)CO-$, -CH=C(CN)CO-, -CH(OH)-NH-, -CH(CN)-NH-, $-CH(OH)-CH_2-$ or -NH-CHOH-, where X is H or an amino protecting group and Y is H or halogen, especially F. Preferred non-natural peptide linkages are $-CO_2-$ or $-CH_2O-$.

Metternich (EP 471651 and US 5288707, the latter being assigned to Trigen Limited) discloses variants of Phe-Pro-BoroArg boropeptides in which the P3 Phe is replaced by an unnatural hydrophobic amino acid such as trimethylsilylalanine, p-tert.butyl-diphenyl-silyloxymethyl-phenylalanine or p-hydroxymethylphenylalanine and the P1 side chain may be neutral (alkoxyalkyl, alkylthioalkyl or trimethylsilylalkyl).

Amparo (WO 96/20698 and family members including US 5698538) discloses peptidomimetics of the structure Aryl-linker-Boro(Aa), where Boro(Aa) may be an aminoboronate residue with a non-basic side chain, for example BoroMpg. The linker is of the formula $-(CH_2)_mCONR-$ (where m is 0 to 8 and R is H or certain organic groups) or analogues thereof in which the peptide linkage -CONR- is replaced by -CSNR-, $-SO_2NR-$, $-CO_2-$, -C(S)O- or $-SO_2O-$. Aryl is phenyl, naphthyl or biphenyl substituted by one, two or three moieties selected from a specified group. Most typically these compounds are of the structure Aryl–$(CH_2)_n$-CONH-CHR^2-BY^1Y^2, where R^2 is for example a neutral side chain as described above and n is 0 or 1.

Non-peptide boronates have been proposed as inhibitors of proteolytic enzymes in detergent compositions. WO 92/19707 and WO 95/12655 report that arylboronates can be used as inhibitors of proteolytic enzymes in detergent compositions. WO 92/19707 discloses compounds substituted *meta* to the boronate group by a hydrogen bonding group, especially acetamido ($-NHCOCH_3$), sufonamido ($-NHSO_2CH_3$) and alkylamino. WO 95/12655 teaches that *ortho*-substituted compounds are superior.

Boronate enzyme inhibitors have wide application, from detergents to bacterial sporulation inhibitors to pharmaceuticals. In the pharmaceutical field, there is ample patent literature describing boronate inhibitors of serine proteases, for example thrombin, factor Xa, kallikrein, elastase, plasmin as well as other serine proteases like prolyl endopeptidase and Ig AI Protease. Thrombin is the last protease in the coagulation pathway and acts to hydrolyse four small peptides form each molecule of fibrinogen, thus deprotecting its polymerisation sites. Once formed, the linear fibrin polymers may be cross-linked by factor XIIIa, which is itself activated by thrombin. In addition, thrombin is a potent activator of platelets, upon which it acts at specific receptors. Thrombin also potentiates its own production by the activation of factors V and VIII.

Other aminoboronate or peptidoboronate inhibitors or substrates of serine proteases are described in:

- US 4935493
- EP 341661
- WO 94/25049
- WO 95/09859
- WO 96/12499
- WO 96/20689
- Lee S-L et al, *Biochemistry* **1997**; *36*, 13180-13186
- Dominguez C et al, *Bioorg. Med. Chem. Lett.* **1997**; *7*, 79-84
- EP 471651
- WO 94/20526
- WO 95/20603
- WO97/05161
- US 4450105
- US 5106948
- US 5169841.

Peptide boronic acid inhibitors of hepatic C virus protease are described in WO 01/02424.

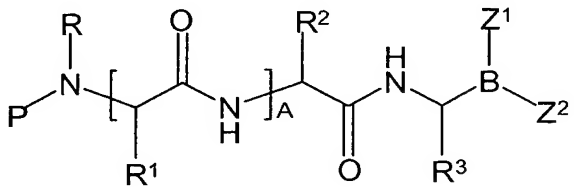
Boronic acid and ester compounds have displayed promise as inhibitors of the proteasome, a multicatalytic protease responsible for the majority of intracellular protein turnover. Ciechanover, *Cell*, **79**: 13-21 (1994), teaches that the proteasome is the proteolytic component of the ubiquitin-proteasome pathway, in which proteins are targeted for degradation by conjugation to multiple molecules of ubiquitin. Ciechanover also teaches that the ubiquitin-proteasome pathway plays a key role in a variety of important physiological processes.

Adams et al, US Patent No 5780454 (1998), US Patent No 6066730 (2000), US Patent No 6083903 (2000) and equivalent WO 96/13266, and US Patent No 6297217 (2001), hereby incorporated by reference in their entirety, describe peptide boronic ester and acid compounds useful as proteasome inhibitors. The references also describe the use of boronic ester and acid compounds to reduce the rate of muscle protein degradation, to reduce the activity of NF-κB in a cell, to reduce the rate of degradation of p53 protein in a cell, to inhibit cyclin degradation in a cell, to inhibit the growth of a cancer cell, to inhibit antigen presentation in a cell, to inhibit NF-κB dependent cell adhesion, and to inhibit HIV replication. Brand et al, WO 98/35691, teaches that proteasome inhibitors, including boronic acid compounds, are useful for treating infarcts such as occur during stroke or myocardial infarction. Elliott et al, WO 99/15183, teaches that proteasome inhibitors are useful for treating inflammatory and autoimmune diseases.

Unfortunately, organoboronic acids can be relatively difficult to obtain in analytically pure form. For example, Snyder et al, *J. Am. Chem Soc.* 80: 3611 (1958), teaches that arylboronic acid compounds readily form cyclic trimeric anhydrides under dehydrating conditions. Also, alkylboronic acids and their boroxines are often air-sensitive. Korcek et al, *J. Chem. Soc. Perkin Trans.* 2 242 (1972), teaches that butylboronic acid is readily oxidized by air to generate 1-butanol and boric acid.

Wu et al, *J. Pharm. Sci.*, 89: 758-765 (2000), discuss the stability of the compound N-(2-pyrazine) carbonyl-phenylalanine-leucine boronic acid (LDP-341, also known as bortezomib), an anti-cancer agent. It is described how "during an effort to formulate [LDP-341] for parenteral administration, the compound showed erratic stability behaviour". The degradation pathways were investigated and it was concluded that the degradation was oxidative, the initial oxidation being attributed to peroxides or molecular oxygen and its radicals.

WO 02/059131 claims boronic acid products which are described as stable. In particular, these products are certain boropeptides and/or boropeptidomimetics in which the boronic acid group has been derivatised with a sugar. The claimed sugar derivatives, which have hydrophobic amino acid side chains, are of the formula



wherein:

P is hydrogen or an amino-group protecting moiety;

R is hydrogen or alkyl;

A is 0, 1 or 2;

R^1, R^2 and R^3 are independently hydrogen, alkyl, cycloalkyl, aryl or $-CH_2-R^5$;

R^5, in each instance, is one of aryl, aralkyl, alkaryl, cycloalkyl, heterocyclyl, heteroaryl, or -W-R^6, where W is a chalcogen and R^6 is alkyl;

where the ring portion of any of said aryl, aralkyl, alkaryl, cycloalkyl, heterocyclyl, or heteroaryl in R^1, R^2, R^3 or R^5 can be optionally substituted; and

Z^1 and Z^2 together form a moiety derived from a sugar, wherein the atom attached to boron in each case is an oxygen atom.

Some of the claimed compounds are sugar derivatives of LDP-341 (see above).

Many drugs comprise an active moiety which is a carboxylic acid. There are a number of differences between carboxylic acids and boronic acids, whose effects on drug delivery, stability and transport (amongst others) have not been investigated. One feature of trivalent boron compounds is that the boron atom is sp^2 hybridised, which leaves an empty $2p_z$ orbital on the boron atom. A molecule of the type BX_3 can therefore act as an electron-pair acceptor, or Lewis acid. It can use the empty $2p_z$ orbital to pick up a pair of nonbonding electrons from a Lewis base to form a covalent bond. BF_3 therefore reacts with Lewis bases such as NH_3 to form acid-base complexes in which all of the atoms have a filled shell of valence electrons.

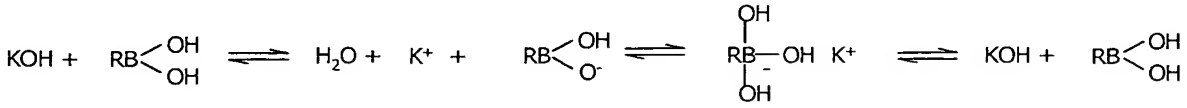
Boric acid, accordingly, can act as a Lewis acid, accepting OH^-:

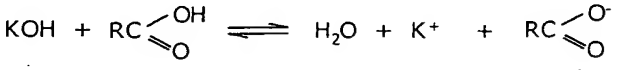
$$B(OH)_3 + H_2O \rightarrow B(OH)_4^- + H^+$$

Further, boronic acids of the type $RB(OH)_2$ are dibasic and have two pKa's. Another point of distinction about boron compounds is the unusually short length of bonds to boron, for which three factors may be responsible:

1. Formation of $p\pi$-$p\pi$ bonds;
2. Ionic-covalent resonance;
3. Reduced repulsions between non-bonding electrons.

The presumed equilibria of boronic and carboxylic acids in aqueous KOH are shown below (excluding formation of RBO_2^{2-}):





Thrombosis

Hemostasis is the normal physiological process in which bleeding from an injured blood vessel is arrested. It is a dynamic and complex process in which proteolytic enzymes such as thrombin play a key role. Blood coagulation may occur through either of two cascades of zymogen activations, the extrinsic and intrinsic pathways of the coagulation cascade. Factor VIIa in the extrinsic pathway, and Factor IXa in the intrinsic pathway are important determinants of the activation of factor X to factor Xa, which itself catalyzes the activation of prothrombin to thrombin. The last protease in each pathway is thrombin, which acts to hydrolyze four small peptides (two FpA and two FpB) from each molecule of fibrinogen, thus deprotecting its polymerization sites. Once formed, the linear fibrin polymers may be cross-linked by factor XIIIa, which is itself activated by thrombin. In addition, thrombin is a potent activator of platelets, upon which it acts at specific receptors. Thrombin activation of platelets leads to aggregation of the cells and secretion of additional factors that further accelerate the creation of a hemostatic plug. Thrombin also potentiates its own production by the activation of factors V and VIII (see Hemker and Beguin in: Jolles, et. al., "Biology and Pathology of Platelet Vessel Wall Interactions," pp. 219-26 (1986), Crawford and Scrutton in: Bloom and Thomas, "Haemostasis and Thrombosis," pp. 47-77, (1987), Bevers, et. al., *Eur. J. Biochem.* **1982,** 122, 429-36, Mann, *Trends Biochem. Sci.* **1987,** 12, 229-33).

Proteases are enzymes which cleave proteins at specific peptide bonds. Cuypers et al., *J. Biol. Chem.* 257:7086 (1982), and the references cited therein, classify proteases on a mechanistic basis into five classes: serine, cysteinyl or thiol, acid or aspartyl, threonine and metalloproteases. Members of each class catalyse the hydrolysis of peptide bonds by a similar mechanism, have similar active site amino acid residues and are susceptible to class-specific inhibitors. For example, all serine proteases that have been characterised have an active site serine residue.

The coagulation proteases thrombin, factor Xa, factor VIIa, and factor IXa are serine proteases having trypsin-like specificity for the cleavage of sequence-specific Arg-Xxx peptide bonds. As with other serine proteases, the cleavage event begins with an attack of the active site serine on the scissile bond of the substrate, resulting in the formation of a tetrahedral intermediate. This is followed by collapse of the tetrahedral intermediate to form an acyl enzyme and release of the amino terminus of the cleaved sequence. Hydrolysis of the acyl enzyme then releases the carboxy terminus.

As indicated above, platelets play two important roles in normal hemostasis. First, by aggregating, they constitute the initial hemostatic plug which immediately curtails bleeding from broken blood vessels. Secondly, the platelet surface can become activated and potentiate blood clotting, a property referred to as platelet procoagulant activity. This may be observed as an increase in the rate of activation of prothrombin by factor Xa in the presence of factor Va and Ca^{2+}, referred to as the prothrombinase reaction. Normally, there are few (if any) clotting factors on the surface of

unstimulated platelets but, when platelets are activated, negatively charged phospholipids (phosphatidylserine and phospatidylinositol) that are normally on the cytoplasmic side of the membrane become available and provide a surface on which two steps of the coagulation sequence occur. The phospholipid on the surface of activated platelets profoundly accelerates the reactions leading to the formation of thrombin, so that thrombin can be generated at a rate faster than its neutralisation by antithrombin III. The reactions that occur on the platelet surfaces are not easily inhibited by the natural anticoagulants in blood such as antithrombin III, either with or without heparin. (See Kelton and Hirsch in : Bloom and Thomas, "Haemostasis and Thrombosis," pp. 737-760, (1981); Mustard et al in : Bloom and Thomas, "Haemostasis and Thrombosis," pp. 503526, (1981); Goodwin et al; *Biochem. J.* **1995**, *308*, 15-21).

A thrombus can be considered as an abnormal product of a normal mechanism and can be defined as a mass or deposit formed from blood constituents on a surface of the cardiovascular system, for example of the heart or a blood vessel. Thrombosis can be regarded as the pathological condition wherein improper activity of the hemostatic mechanism results in intravascular thrombus formation. Three basic types of thrombi are recognised:

- the white thrombus which is usually seen in arteries and consists chiefly of platelets;
- the red thrombus which is found in veins and is composed predominantly of fibrin and red cells;
- the mixed thrombus which is composed of components of both white and red thrombi.

The composition of thrombi is influenced by the velocity of blood flow at their sites of formation. In general white platelet-rich thrombi form in high flow systems, while red coagulation thrombi form in regions of stasis. The high shear rate in arteries prevents the accumulation of coagulation intermediates on the arterial side of the circulation: only platelets have the capacity to form thrombi binding to the area of damage via von Willebrand factor. Such thrombi composed only of platelets are not stable and disperse. If the stimulus is strong then the thrombi will form again and then disperse continually until the stimulus has diminished. For the thrombus to stabilise, fibrin must form. In this respect, small amounts of thrombin can accumulate within the platelet thrombus and activate factor Va and stimulate the platelet procoagulant activity. These two events lead to an overall increase in the rate of activation of prothrombin by factor Xa of 300,000 fold. Fibrin deposition stabilises the platelet thrombus. Thrombin inhibitors are not clinically effective at inhibiting stimulation of platelet procoagulant activity. Accordingly, a therapeutic agent which inhibits platelet procoagulant activity would be useful for treating or preventing arterial thrombotic conditions

On the venous side of circulation, the thrombus is comprised of fibrin: thrombin can accumulate because of the slower flow on the venous side and platelets play only a minor role.

Thrombosis is thus not considered to be a single indication but, rather, is a class of indications embracing distinct sub-classes for which differing therapeutic agents and/or protocols may be

appropriate. Thus, regulatory authorities treat disorders such as, for example, deep vein thrombosis, cerebrovascular arterial thrombosis and pulmonary embolism as distinct indications for the purposes of licensing medicines. Two main sub-classes of thrombosis are arterial thrombosis and venous thrombosis. Arterial thrombosis includes such specific disorders as acute coronary syndromes [for example acute myocardial infarction (heart attack, caused by thrombosis in a coronary artery)], cerebrovascular arterial thrombosis (stroke, caused by thrombosis in the cerebrovascular arterial system) and peripheral arterial thrombosis. Examples of conditions caused by venous thrombosis are deep vein thrombosis and pulmonary embolism.

The management of thrombosis commonly involves the use of thrombolytic agents in combination with anticoagulants and antiplatelet drugs (inhibitors of platelet aggregation) to lyse the newly formed clot and to control future thrombogenesis. Anticoagulants are used also in the treatment of patients thought susceptible to thrombosis.

Currently, two of the most effective classes of drugs in clinical use as anticoagulants are the heparins and the vitamin K antagonists. The heparins are ill-defined mixtures of sulfated polysaccharides that bind to, and thus potentiate, the action of antithrombin III. Antithrombin III is a naturally occurring inhibitor of the activated clotting factors IXa, Xa, XIa, thrombin and probably XIIa (see Jaques, *Pharmacol. Rev.* **1980**, 31, pp. 99-166). The vitamin K antagonists, of which warfarin is the most well-known example, act indirectly by inhibiting the post-ribosomal carboxylations of the vitamin K dependent coagulation factors II, VII, IX and X (see Hirsch, *Semin. Thromb. Hemostasis* **1986**, 12, 1-11). While effective therapies for the treatment of thrombosis, heparins and vitamin K antagonists have the unfortunate side effects of bleeding, heparin-induced thrombocytopenia (in the case of heparin) and marked interpatient variability, resulting in a small and unpredictable therapeutic safety margin.

The use of direct acting inhibitors of thrombin and other serine protease enzymes of the coagulation system is expected to alleviate these problems. To that end, a wide variety of serine protease inhibitors have been tested, including boropeptides, i.e. peptides containing a boronic acid analogue of an α-amino acid. Whilst direct acting boronic acid thrombin inhibitors have been discussed earlier in this specification, they are further described in the following paragraph.

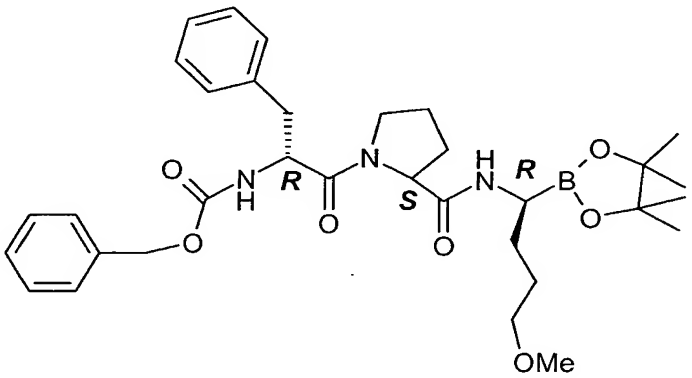
Neutral P1 Residue Boropeptide Thrombin Inhibitors

Claeson et al (US 5574014 and others) and Kakkar et al (WO 92/07869 and family members including US 5648338) disclose lipophilic thrombin inhibitors having a neutral (uncharged) C-terminal (P1) side chain, for example an alkoxyalkyl side chain. The aforementioned US patents of Claeson et al and Kakkar et al (US 5574014 and US 5648338) are incorporated herein by reference.

The Claeson et al and Kakkar et al patent families disclose boronate esters containing the amino acid sequence D-Phe-Pro-BoroMpg [(R)-Phe-Pro-BoroMpg], which are highly specific inhibitors of thrombin. Of these compounds may be mentioned in particular Cbz-(R)-Phe-Pro-BoroMpg-OPinacol (also known as TRI 50b). The corresponding free boronic acid is known as TRI 50c. For further information relating to TRI 50b and related compounds, the reader is referred to the following documents, all incorporated herein by reference:

- Elgendy S et al., in *The Design of Synthetic Inhibitors of Thrombin*, Claeson G et al Eds, *Advances in Experimental Medicine*, **1993,** *340*, pp, pp 173-178.
- Claeson G et al, *Biochem J.* **1993**, *290*, 309-312
- Tapparelli C et al, *J Biol Chem*, **1993**, *268*, 4734-4741
- Claeson G, in *The Design of Synthetic Inhibitors of Thrombin*, Claeson G et al Eds, *Advances in Experimental Medicine*, **1993,** *340*, pp 83-91
- Phillip et al, in *The Design of Synthetic Inhibitors of Thrombin*, Claeson G et al Eds, *Advances in Experimental Medicine*, **1993,** *340*, pp 67-77
- Tapparelli C et al, *Trends Pharmacol. Sci.* **1993**, *14*, 366-376
- Claeson G, *Blood Coagulation and Fibrinolysis* **1994**, *5*, 411-436
- Elgendy et al, *Tetrahedron* **1994**, *50*, 3803-3812
- Deadman J et al, *J. Enzyme Inhibition* **1995**, *9*, 29-41.
- Deadman J et al, *J. Medicinal Chemistry* **1995**, *38*, 1511-1522.

The tripeptide sequence of TRI 50b has three chiral centres. The Phe residue is considered to be of R (= D) configuration and the Pro residue of natural S (= L) configuration, at least in compounds with commercially useful inhibitor activity; the Mpg residue is believed to be of R (= L) configuration in isomers with commercially useful inhibitor activity. Thus, the active, or most active, TRI 50b stereoisomer is considered to be of RSR configuration and may be represented as:



(RSR)-TRI 50b: Cbz-(R)-Phe-(S)-Pro-(R)-boroMpg Pinacol

Whilst direct acting thrombin inhibitors have been found useful for the treatment of patients susceptible to or suffering from venous thrombosis, the same is not true of arterial thrombosis. In

the case of currently available thrombin inhibitors, it would be necessary to raise the dosage used in the treatment of venous thrombosis by many times in order to treat (prevent) arterial thrombosis. Such raised dosages typically cause bleeding, which makes direct acting thrombin inhibitors unsuitable for treating arterial thrombosis. Heparin, which primarily acts as a thrombin inhibitor, is also unsuitable to treat arterial thrombosis. It has been found that a class of compounds which is defined by Formula (III) below and represented by boropeptides having the amino acid sequence (R)-Phe-Pro-BoroMpg is beneficial in that the members of the class are useful for treating arterial thrombosis by therapy or prophylaxis.

Oral Absorption

Absorption in the gastro-intestinal tract can be by an active or a passive route. Active absorption by transport mechanisms tends to be variable between individuals and with intestinal content (Gustafsson et al, *Thrombosis Research*, **2001**, *101*, 171-181). The upper intestine has been identified as the principal site of oral drug absorption. In particular, the duodenum is the customary target site for absorption of orally administered drugs because of its large surface area. The intestinal mucosa acts as a barrier that controls passive transcellular absorption: the absorption of ionic species is blocked whilst the transcellular absorption of lipophilic molecules is favoured (Palm K et al., *J. Pharmacol and Exp. Therapeutics*, **1999**, *291*, 435-443).

Orally administered drugs are required to be consistently and adequately absorbed. Variability of absorption between individuals or between different occasions in the same individual is unwelcome. Similarly, drugs which have a low level of bioavailability (only a small portion of the administered active agent is absorbed) are generally unacceptable.

Non-ionised compounds are favoured for passive absorption, a route associated with invariability, and are therefore preferred for consistent absorption. Lipophilic species are particularly favoured by passive absorption mechanisms and, accordingly, non-ionic, lipophilic drugs are indicated to be most favoured for consistent and high oral absorption.

Typical functionalities required for interaction of drugs with their physiological targets are functional groups such as carboxylic and sulphonic acids. These groups exist as the protonated form in the stomach (at pH 2-3), but will be ionised to some extent at the higher pH of the intestinal fluid. One strategy that has been used to avoid the ionisation of the carboxylates or sulphonates is to present them as ester forms, which are cleaved once absorbed into the vascular lumen.

For example, the direct acting thrombin inhibitor melagatran, which has sub-optimal gastrointestinal absorption, has terminal carboxy and amidino groups and is a pure zwitterion at pH 8-10 when the carboxylic acid and amidino groups are both charged. A prodrug H 376/95 was therefore developed

which has protecting groups for the carboxylic acid and for the amidine and is a more lipophilic molecule than melagatran. The prodrug has a permeability coefficient across cultured epithelial Caco-2 cells 80 times higher than that of melagatran and oral bioavailability 2.7-5.5 times higher than that of melagatran as well as much smaller variability in the area under the drug plasma concentration vs. time curve (Gustafsson et al, *Thrombosis Research*, **2001**, *101*, 171-181).

Oral Absorption of Boropeptides, Boropeptidomimetics and other Organoboronates

The boronate ester group of TRI 50b is rapidly cleaved in the conditions of the plasma to form the corresponding boronic acid group, which is considered to be the active moiety which inhibits the catalytic site of thrombin.

Boronic acids are divalent functional groups, with boron-oxygen bond lengths (1.6Å) more typical of single bonds, unlike superficially comparable C-O and S-O bonds in carboxylic and sulphonic acids. Consequently the boronic acid group has two ionisation potentials. The boronic acid group will be partly ionised at pH's of the duodenal fluid and not suited to the desired passive duodenal uptake. Thus, a charged boronate inhibitor H-D-PheProBoroArg is absorbed by a predominantly active transport mechanism (Saitoh, H. and Aungst, B.J., *Pharm. Res.*, **1999**, *16*, 1786-1789).

The peptide boronic acid formed by such cleavage of TRI 50b (the acid is designated TRI 50c) is relatively insoluble in water, especially at acidic or neutral pH, and tends to be poorly absorbed in the stomach and duodenum. The acid has the structure Cbz-Phe-Pro-BoroMpg-OH.

Whereas the peptide boronic acid Cbz-Phe-Pro-BoroMpg-OH is partly ionised under duodenal conditions and, to that extent, unfavoured for passive transport, esters of the acid are designed for a high rate of passive (thus consistent) transport. The tripeptide sequence Phe-Pro-Mpg has a non-basic P1 side chain (specifically, methoxypropyl), such that the tripeptide consists of three non-polar amino acids. The esters of the peptide boronic acid are non-ionisable and the ester-forming species further impart lipophilic properties, so encouraging a high rate of passive transport.

Computational techniques have confirmed that TRI 50b and other diol esters of Cbz-Phe-Pro-BoroMpg-OH can be predicted to have good bioavailability. Thus, polar surface area (PSAd) is a parameter predictive of bioavailability and PSAd values of greater than 60Å correlate well with passive transcellular transport and with bioavailability of known drugs (Kelder, *J. Pharm. Res.*, **1999**, *16*, 1514-1519). Measurements for diol esters of the above peptide boronic acid, including the pinacol ester TRI 50b, show that the diol esters have PSAd values well above 60Å, predictive of passive transport and good bioavailability as shown in Table 1:

Table 1: PSAd values of selected di l esters of Cbz-Phe-Pro-B r Mpg-OH

Diol	PSAd Value
Pinacol	98.74
Pinanediol	90.64

The corresponding monohydroxy alcohol (e.g. alkanol) esters were considered too unstable, spontaneously cleaving to liberate the acid *in-vitro*. Esters of diols such as pinanediol and pinacol have enhanced kinetic stability over esters of monohydroxy alcohols, in that after partial hydrolysis to the mono-ester derivative they will tend to reassociate by a facile intra-molecular reaction.

To counterbalance these highly desirable features of TRI 50b, it has been discovered that TRI 50b tends to hydrolyse. Thus in the acid conditions of an HPLC assay, TRI 50b is converted to the acid form with a short half life, which implies potential intraduodenal hydrolysis into ionic species which would resist passive transport and, if anything, be absorbed by active transport, indicative at best of variable bioavailability.

The instability of TRI 50b to hydrolysis also presents potential disadvantages in preparation of the compound and its formulation, as well as in the storage of pharmaceutical formulations containing it.

Another challenging difficulty which has been posed by TRI 50b is that the data show significant variation in bioavailability between subjects. Such variability can make a drug candidate unacceptable and it would therefore be desirable to reduce the observed variability.

An ideal solution to the instability of TRI 50b would be development of a diol ester more stable to hydrolysis. In this regard, it is known that ring size can affect boronate stability and glycolato boron has been shown to have enhanced aqueous stability compared to pinacol (D.S.Matteson, Stereodirected Synthesis with Organoboranes, Springer-Verlag, 1995, ch.1). Similarly, the pinanediol ester is more stable than the pinacol; this is believed to be because the pinanediol group is highly sterically hindered and disfavours nucleophilic attack on the boron. In fact transesterification from pinacol to pinanediol has been reported (Brosz, CS, *Tet. Assym*, **1997**, *8*, 1435-1440) whereas the reverse process is unfavourable. The pinanediol ester however is considered too slow to cleave in plasma and there remains a need to provide an improved diol ester.

Another solution to the instability of TRI 50b would be to administer in its place TRI 50c. However, TRI 50c data suggest that TRI 50c too suffers from variability in bioavailability.

TRI 50c suffers further from instability, in that there is a problematic tendency for the boropeptide moiety itself to degrade. The level of degradation can be remarkably high.

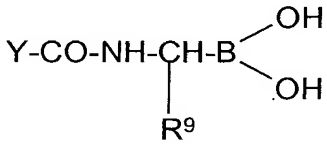
The present invention provides a different solution to the problem of boronate diol ester and especially TRI50b instability. It further provides derivatives of TRI 50b/TRI 50c wherein the boropeptide moiety is indicated to be of enhanced stability.

The properties discussed above of TRI 50b and TRI 50c will not be restricted to such compounds but will be shared by other boropeptide esters and acids, even if the properties of such other boropeptides differ quantitatively.

BRIEF SUMMARY OF THE INVENTION

The invention provides an amino boronic acid derivative which, in the conditions of the duodenum, contradictorily releases an ionic boropeptide species whilst avoiding the disadvantages of pinacol esters as well as enabling consistent and adequate bioavailability. It further includes a peptide boronic acid derivative which is indicated to be of enhanced stability.

In one aspect, the invention provides salts of boronic acids which have a neutral aminoboronic acid residue capable of binding to the thrombin S1 subsite linked through a peptide linkage to a hydrophobic moiety capable of binding to the thrombin S2 and S3 subsites. The acid may for example be of formula (I):



(I)

wherein

Y comprises a hydrophobic moiety which, together with the aminoboronic acid residue $-NHCH(R^9)-B(OH)_2$, has affinity for the substrate binding site of thrombin; and

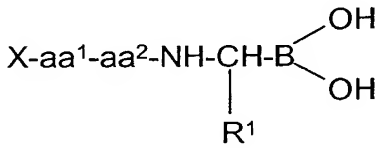
R^9 is a straight chain alkyl group interrupted by one or more ether linkages (e.g. 1 or 2) and in which the total number of oxygen and carbon atoms is 3, 4, 5 or 6 (e.g. 5) or R^9 is $-(CH_2)_m$-W where m is 2, 3, 4 or 5 (e.g. 4) and W is –OH or halogen (F, Cl, Br or I). R^9 is an alkoxyalkyl group in one subset of compounds, e.g. alkoxyalkyl containing 4 carbon atoms.

Such salts are not only contrary to the direction of the prior art but additionally have an improved level of stability which cannot be explained or predicted on the basis of known chemistry.

The invention comprises salts of hydrophobic boronic acid inhibitors of thrombin, and therefore includes salts of peptide boronic acids which have a partition coefficient between 1-n-octanol and

water expressed as log P of greater than 1.0 at physiological pH and 25°C. Some peptide boronic acids useful in the invention have a partition coefficient of at least 1.5. A class of hydrophobic peptide boronic acids useful in the invention has a partition coefficient of no more than 5.

The present invention includes a salt of a peptide boronic acid of formula (II):



(II)

where:

X is H (to form NH_2) or an amino-protecting group;

aa^1 is an amino acid having a hydrocarbyl side chain containing no more than 20 carbon atoms (e.g. up to 15 and optionally up to 13 C atoms) and comprising at least one cyclic group having up to 13 carbon atoms;

aa^2 is an imino acid having from 4 to 6 ring members; and

R^1 is a group of the formula $-(CH_2)_s-Z$, where s is 2, 3 or 4 and Z is –OH, –OMe, –OEt or halogen (F, Cl, Br or I). Alternatively, R^1 may be replaced by a side chain R^9 as defined above.

Preferably, the cyclic group(s) of aa^1 is/are aryl groups, particularly phenyl. More preferably, aa^1 is Phe, Dpa or a wholly or partially hydrogenated analogue thereof.

These salts are stable to hydrolysis and are indicated to be more stable to degradation of the organoboronate species than are the corresponding acids. Further, the invention comprises salts which are more soluble than corresponding peptide boronic acids and their esters.

There is a debate in the literature as to whether boronates in aqueous solution form the 'trigonal' $B(OH)_2$ or 'tetrahedral' $B(OH)_3^-$ boron species, but NMR evidence seems to indicate that at a pH below the first pKa of the boronic acid the main boron species is the neutral $B(OH)_2$. In the duodenum the pH is likely to be between 6 and 7, so the trigonal species is likely to be predominant here. In any event, the symbol $-B(OH)_2$ includes tetrahedral as well as trigonal boron species, and throughout this specification symbols indicating trigonal boron species embrace also tetrahedral species.

The salts may be in the form of solvates, particularly hydrates.

The salts may comprise, or consist essentially of, acid salts. The invention therefore includes products having a metal/boronate stoichiometry consistent with the boronate groups in the product predominantly (more than 50 mol %) carrying a single negative charge.

The invention includes also oral formulations of the salts of the invention.

According to a further aspect of the present invention, there is provided a method of treatment of a condition where anti-thrombotic activity is required which method comprises oral administration of a therapeutically effective amount of a salt of a boronic acid of formula (I) to a person suffering from, or at risk of suffering from, such a condition.

The salts described herein are obtainable by (have the characteristics of a product obtained by) reaction of the boronic acid with a strong base and the term "salt" herein is to be understood accordingly. The term "salt" in relation to the products of the invention, therefore, does not necessarily imply that the products contain discrete cations and anions and is to be understood as embracing products which are obtainable using a reaction of a boronic acid and a base. The invention thus provides also products obtainable by (having the characteristics of a product obtained by) reaction of a boronic acid (I) with a strong base a well as the therapeutic, including prophylactic, use of such products.

The invention is not limited as to the method of preparation of the salts, provided that they contain a boronate species derived from boronic acid (I) and a counter-ion. It is not required that the salts be prepared by reaction of a base containing the counter-ion and the boronic acid (I). Further, the invention includes salt products which might be regarded as indirectly prepared by such an acid/base reaction as well as salts obtainable by (having the characteristics of a products obtained by) such indirect preparation. As examples of possibly indirect preparation may be mentioned processes in which, after initial recovery of the salt, it is purified and/or treated to modify its physicochemical properties, for example to modify solid form or hydrate form, or both.

In some embodiments, the cations of the salts are monovalent.

The salts may be in isolated form. The salts may have a purity of at least 90%, e.g. of greater than or equal to 95%, for example purities of up to 99.5%. In the case of pharmaceutical formulations, such salt forms may be combined with pharmaceutically acceptable diluents, excipients or carriers.

The invention includes a method for preparing the salts from the corresponding boronic acid as an intermediate, as well as the intermediate boronic acid of Formula (I) and a method for preparing it.

Further aspects and embodiments of the invention are set forth in the following description and claims.

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of the words, for example "comprising" and "comprises", mean "including but not limited to", and are not intended to (and do not) exclude other moieties, additives, components, integers or steps.

This patent application contains data indicating that the stability of organoboronic acids may be increased by providing them in the form of salts, e.g. metal salts. The salt may be an acid salt. This technique forms part of the invention and is applicable, *inter alia*, to organoboronic acids described under the heading "BACKGROUND OF THE INVENTION" and to organoboronic acids described in publications mentioned under that heading.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an HPLC plot referred to in Example 3, showing the chromatographic separation of:

a) TRI50b(I), the RSR stereoisomer of TRI 50b (retention time = 11.1 min),
b) TRI50b(II), the RSS stereoisomer of TRI 50b (retention time = 13.7 min),
c) TRI50c(I), the RSR stereoisomer of TRI 50c (retention time = 21.2 min),
d) TRI50c(II), the RSS stereoisomer of TRI 50c (retention time = 22.2 min).

Figure 2 is a plot referred to in Example 32, showing intravenous phase clearance and kinetics following a single dose of TRI 50b or TRI 50c.

Figure 3 is a second plot referred to in Example 32, showing oral phase clearance and kinetics following p.o. dosing with TRI 50b or TRI 50c.

Figure 4 is a third plot referred to in Example 32, showing oral phase clearance and kinetics following intraduodenal dosing with TRI 50b or TRI 50c.

DETAILED DESCRIPTION OF THE INVENTION

Gl ssary

The following terms and abbreviations are used in this specification:

The expression "acid salt" as applied to a salt of a boronic acid refers to salts of which a single -OH group of the trigonally-represented acid group $-B(OH)_2$ is deprotonated. Thus salts wherein the boronate group carries a single negative charge and may be represented as $-B(OH)(O^-)$ or as $[-B(OH)_3]^-$ are acid salts. The expression encompasses salts having a cation having a valency V wherein the molar ratio of boronic acid to cation is approximately V to 1. In practical terms, the observed stoichiometry is unlikely to be exactly V:1 but will be consistent with a notional V:1 stoichiometry. For example, the observed mass of the cation might vary from the calculated mass for a V:1 stoichiometry by no more than about 10%, e.g. no more than about 7.5%; in some cases an observed mass of a cation might vary from the calculated mass by no more than about 1%. Calculated masses are suitably based on the trigonal form of the boronate. (At an atomic level, a salt stoichiometrically consistent with being an acid salt might contain boronates in a mix of protonation states, whose average approximates to single deprotonation and such "mixed" salts are included in the term "acid salt").

α-Aminoboronic acid or Boro(aa) refers to an amino acid in which the CO_2 group has been replaced by BO_2.

The term "amino-group protecting moiety" refers to any group used to derivatise an amino group, especially an N-terminal amino group of a peptide or amino acid. Such groups include, without limitation, alkyl, acyl, alkoxycarbonyl, aminocarbonyl, and sulfonyl moieties. However, the term "amino-group protecting moiety" is not intended to be limited to those particular protecting groups that are commonly employed in organic synthesis, nor is it intended to be limited to groups that are readily cleavable.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings or animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The expression "thrombin inhibitor" refers to a product which, within the scope of sound pharmacological judgement, is potentially or actually pharmaceutically useful as an inhibitor of thrombin, and includes reference to substance which comprises a pharmaceutically active species and is described, promoted or authorised as a thrombin inhibitor. Such thrombin inhibitors may be selective, that is they are regarded, within the scope of sound pharmacological judgement, as selective towards thrombin in contrast to other proteases; the term "selective thrombin inhibitor"

includes reference to substance which comprises a pharmaceutically active species and is described, promoted or authorised as a selective thrombin inhibitor.

The term "heteroaryl" refers to a ring system which has at least one (e.g. 1, 2 or 3) in-ring heteroatoms and has a conjugated in-ring double bond system. The term "heteroatom" includes oxygen, sulfur and nitrogen, of which sulfur is sometimes less preferred.

"Natural amino acid" means an L-amino acid (or residue thereof) selected from the following group of neutral (hydrophobic or polar), positively charged and negatively charged amino acids:

Hydrophobic amino acids
A = Ala = alanine
V = Val = valine
I = Ile = isoleucine
L = Leu = leucine
M = Met = methionine
F = Phe = phenylalanine
P = Pro = proline
W = Trp = tryptophan

Polar (neutral or uncharged) amino acids
N = Asn = asparagine
C = Cys = cysteine
Q = Gln = glutamine
G = Gly = glycine
S = Ser = serine
T = Thr = threonine
Y = Tyr = tyrosine

Positively charged (basic) amino acids
R = Arg = arginine
H = His = histidine
K = Lys = lysine

Negatively charged amino acids
D = Asp = aspartic acid
E = Glu = glutamic acid.

Amino acid = α-amino acid

Cbz = benzyloxycarbonyl

Cha = cyclohexylalanine (a hydrophobic unnatural amino acid)

Charged (as applied to drugs or fragments of drug molecules, e.g. amino acid residues) = carrying a charge at physiological pH, as in the case of an amino, amidino or carboxy group

Dcha = dicyclohexylalanine (a hydrophobic unnatural amino acid)

Dpa = diphenylalanine (a hydrophobic unnatural amino acid)

Drug = a pharmaceutically useful substance, whether the active in vivo principle or a prodrug

Mpg = 3-methoxypropylglycine (a hydrophobic unnatural amino acid)

Multivalent = valency of at least two, for example two or three

Neutral (as applied to drugs or fragments of drug molecules, e.g. amino acid residues) = uncharged = not carrying a charge at physiological pH

Pinac = Pinacol = 2,3-dimethyl-2,3-butanediol

Pinanediol = 2,3-pinanediol = 2,6,6-trimethylbicyclo [3.1.1] heptane-2,3-diol

Pip = pipecolinic acid

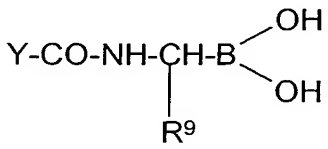
Strong base = a base having a sufficiently high pKb to react with a boronic acid. Suitably such bases have a pKb of 7 or more, e.g. 7.5 or more, for example about 8 or more

THF = tetrahydrofuran

Thr = thrombin

The Compounds

The products of the invention comprise salts of boronic acids which have a neutral aminoboronic acid residue capable of binding to the thrombin S1 subsite linked through a peptide linkage to a hydrophobic moiety capable of binding to the thrombin S2 and S3 subsites. The invention includes salts of acids of formula (I):



(I)

wherein

Y comprises a hydrophobic moiety which, together with the aminoboronic acid residue $-NHCH(R^9)-B(OH)_2$, has affinity for the substrate binding site of thrombin; and

R^9 is a straight chain alkyl group interrupted by one or more ether linkages and in which the total number of oxygen and carbon atoms is 3, 4, 5 or 6 (e.g. 5) or R^9 is $-(CH_2)_m$-W where m is from 2, 3, 4 or 5 (e.g. 4) and W is –OH or halogen (F, Cl, Br or I). R^9 is an alkoxyalkyl group in one subset of compounds, e.g. alkoxyalkyl containing 4 carbon atoms.

Typically, YCO- comprises an amino acid (whether natural or unnatural) which binds to the S2 subsite of thrombin, the amino acid being N-terminally linked to a moiety which binds the S3 subsite of thrombin.

In one class of Formula (I) acids, YCO- is an optionally N-terminally protected dipeptide residue which binds to the S3 and S2 binding sites of thrombin and the peptide linkages in the acid are optionally and independently N-substituted by a C_1-C_{13} hydrocarbyl group optionally containing in-chain and/or in-ring nitrogen, oxygen or sulfur and optionally substituted by a substituent selected from halo, hydroxy and trifluoromethyl. The N-terminal protecting group, when present, may be a group X as defined above (other than hydrogen). Normally, the acid contains no N-substituted peptide linkages; where there is an N-substituted peptide linkage, the substituent is often 1C to 6C hydrocarbyl, e.g. saturated hydrocarbyl; the N-substituent comprises a ring in some embodiments, e.g. cycloalkyl, and may be cyclopentyl, for example. One class of acids has an N-terminal protecting group (e.g. an X group) and unsubstituted peptide linkages.

Where YCO- is a dipeptide (whether or not N-terminally protected), the S3-binding amino acid residue may be of R configuration and/or the S2-binding residue may of S configuration. The fragment –NHCH(R^9)-B(OH) may of R configuration. The invention is not restricted to chiral centres of these conformations, however.

In one class of compounds, the side chain of P3 (S3-binding) amino acid and/or the P2 (S2-binding) amino acid is a moiety other than hydrogen selected from a group of formula A or B:

$$-(CO)_a-(CH_2)_b-D_c-(CH_2)_d-E \qquad (A)$$

$$-(CO)_a-(CH_2)_b-D_c-C_e(E^1)(E^2)(E^3) \qquad (B)$$

wherein

a is 0 or 1;

e is 1;

b and d are independently 0 or an integer such that (b+d) is from 0 to 4 or, as the case may be, (b+e) is from 1 to 4;

c is 0 or 1;

D is O or S;

E is H, C_1-C_6 alkyl, or a saturated or unsaturated cyclic group which normally contains up to 14 members and preferably is a 5-6 membered ring (e.g. phenyl) or an 8-14 membered fused ring system (e.g. naphthyl), which alkyl or cyclic group is optionally substituted by up to 3 groups (e.g. 1 group) independently selected from C_1-C_6 trialkylsilyl, -R^{13}, -$R^{12}OR^{13}$, -$R^{12}COR^{13}$, -$R^{12}CO_2R^{13}$

and $-R^{12}O_2CR^{13}$, wherein R^{12} is $-(CH_2)_f-$ and R^{13} is $-(CH_2)_gH$ or by a moiety whose non-hydrogen atoms consist of carbon atoms and in-ring heteroatoms and number from 5 to 14 and which contains a ring system (e.g. an aryl group) and optionally an alkyl and/or alkylene group, wherein f and g are each independently from 0 to 10, g preferably being at least 1 (although –OH may also be mentioned as a substituent), provided that (f+g) does not exceed 10, preferably does not exceed 6 and more preferably is 1, 2, 3 or 4, and provided that there is only a single substituent if the substituent is a said moiety containing a ring system, or E is C_1-C_6 trialkylsilyl; and E^1, E^2 and E^3 are each independently selected from $-R^{15}$ and $-J-R^{15}$, where J is a 5-6 membered ring and R^{15} is selected from C_1-C_6 trialkylsilyl, $-R^{13}$, $-R^{12}OR^{13}$, $-R^{12}COR^{13}$, $-R^{12}CO_2R^{13}$ and $-R^{12}O_2CR^{13}$ where R^{12} and R^{13} are, respectively, an R^{12} moiety and an R^{13} moiety as defined above (in some acids where E^1, E^2 and E^3 contain an R^{13} group, g is 0 or 1);

in which moiety of Formula (A) or (B) any ring is carbocyclic or aromatic, or both, and any one or more hydrogen atoms bonded to a carbon atom is optionally replaced by halogen, especially F.

Preferably, a is 0. If a is 1, c is preferably 0. Preferably, (a+b+c+d) and (a+b+c+e) are no more than 4 and are more preferably 1, 2 or 3. (a+b+c+d) may be 0.

Exemplary groups for E, E^1, E^2 and E^3 include aromatic rings such as phenyl, naphthyl, pyridyl, quinolinyl and furanyl, for example; non-aromatic unsaturated rings, for example cyclohexenyl; saturated rings such as cyclohexyl, for example. E may be a fused ring system containing both aromatic and non-aromatic rings, for example fluorenyl. One class of E, E^1, E^2 and E^3 groups are aromatic (including heteroaromatic) rings, especially 6-membered aromatic rings. In some compounds, E^1 is H whilst E^2 and E^3 are not H; in those compounds, examples of E^2 and E^3 groups are phenyl (substituted or unsubstituted) and C_1-C_4 alkyl, e.g. methyl.

In one class of embodiments, E contains a substituent which is C_1-C_6 alkyl, (C_1-C_5 alkyl)carbonyl, carboxy C_1-C_5 alkyl, aryl (including heteroaryl), especially 5-membered or preferably 6-membered aryl (e.g. phenyl or pyridyl), or arylalkyl (e.g. arylmethyl or arylethyl where aryl may be heterocyclic and is preferably 6-membered).

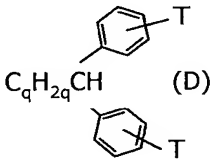
In another class of embodiments, E contains a substituent which is OR^{13}, wherein R^{13} preferably is a 6-membered ring, which may be aromatic (e.g. phenyl) or is alkyl (e.g. methyl or ethyl) substituted by such a 6-membered ring.

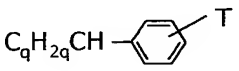
A class of moieties of formula A or B are those in which E is a 6-membered aromatic ring optionally substituted, preferably at the 2-position or 4-position, by $-R^{13}$ or $-OR^{13}$.

The invention includes salts in which the P3 and/or P2 side chain comprises a cyclic group in which 1 or 2 hydrogens have been replaced by halogen, e.g. F or Cl.

The invention includes a class of salts in which the side chains of formula (A) or (B) are of the following formulae (C), (D) or (E):

$C_qH_{2q}CHT_2$ (C)


(D)


(E)

wherein q is from 0 to 5, e.g. is 0, 1 or 2, and each T is independently hydrogen, halogen (e.g. F or Cl), $-SiMe_3$, $-R^{13}$, OR^{13}, $-COR^{13}$, CO_2R^{13} or $-O_2CR^{13}$. In some embodiments of structures (D) and (E), T is at the 4-position of the phenyl group(s) and is $-R^{13}$, $-OR^{13}$, $-COR^{13}$, $-CO_2R^{13}$ or $-O_2CR^{13}$, and R^{13} is C_1-C_{10} alkyl and more preferably C_1-C_6 alkyl. In one sub-class, T is $-R^{13}$ or OR^{13}, for example in which f and g are each independently 0, 1, 2 or 3; in some side chains groups of this sub-class, T is $-R^{12}OR^{13}$ and R^{13} is H.

In one class of the moieties, the side chain is of formula (C) and each T is independently R^{13} or OR^{13} and R^{13} is C_1-C_4 alkyl. In some of these compounds, R^{13} is branched alkyl and in others it is straight chain. In some moieties, the number of carbon atoms is from 1 to 4.

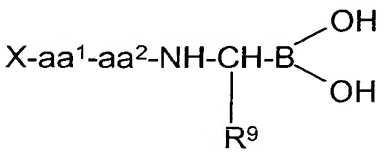
In many dipeptide fragments YCO- (which dipeptides may be N-terminally protected or not), the P3 amino acid has a side chain of formula (A) or (B) as described above and the P2 residue is of an imino acid.

The invention therefore includes medicaments comprising salts, e.g. metal salts, of organoboronic acids which are thrombin inhibitors, particularly selective thrombin inhibitors, having a neutral P1 (S1-binding) moiety. For more information about moieties which bind to the S3, S2 and S1 sites of thrombin, see for example Tapparelli C et al, *Trends Pharmacol. Sci.* **1993**, *14*, 366-376; Sanderson P et al, *Current Medicinal Chemistry*, **1998**, *5*, 289-304; and Rewinkel J et al, *Current Pharmaceutical Design*, **1999**, *5*, 1043-1075. The thrombin inhibitory salts of the invention are not limited to those

having S3, S2 and S1 affinity groups described in the three publications listed in the preceding sentence.

The boronic acids may have a Ki for thrombin of about 100 nM or less, e.g. about 20 nM or less.

A subset of the Formula (I) acids comprises the acids of Formula (III):



(III)

X is a moiety bonded to the N-terminal amino group and may be H to form NH_2. The identity of X is not critical to the invention.

Preferably X is R^6-$(CH_2)_p$-C(O)-, R^6-$(CH_2)_p$-S(O)$_2$-, R^6-$(CH_2)_p$-NH-C(O)- or R^6-$(CH_2)_p$-O-C(O)- wherein p is 0, 1, 2, 3, 4, 5 or 6 (of which 0 and 1 are preferred) and R^6 is H or a 5 to 13-membered cyclic group optionally substituted by 1, 2 or 3 substituents selected from halogen, amino, nitro, hydroxy, a C_5-C_6 cyclic group, C_1-C_4 alkyl and C_1-C_4 alkyl containing, and/or linked to the 5 to 13-membered cyclic group through, an in-chain O, the aforesaid alkyl groups optionally being substituted by a substituent selected from halogen, amino, nitro, hydroxy and a C_5-C_6 cyclic group. More preferably X is R^6-$(CH_2)_p$-C(O)- or R^6-$(CH_2)_p$-O-C(O)- and p is 0 or 1. Said 5 to 13-membered cyclic group is often aromatic or heteroaromatic, for example is a 6-membered aromatic or heteroaromatic group. In many cases, the group is not substituted.

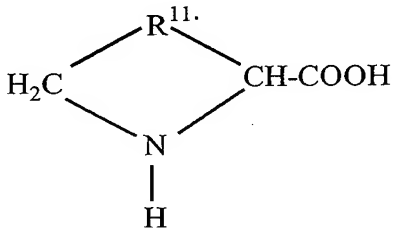
Exemplary X groups are (2-pyrazine) carbonyl, (2-pyrazine) sulfonyl and particularly benzyloxycarbonyl.

aa^1 is an amino acid having a hydrocarbyl side chain containing no more than 20 carbon atoms (e.g. up to 15 and optionally up to 13 C atoms) and comprising at least one cyclic group having up to 13 carbon atoms. Preferably, the cyclic group(s) of aa^1 have/has 5 or 6 ring members. Preferably, the cyclic group(s) of aa^1 is/are aryl groups, particularly phenyl. Typically, there are one or two cyclic groups in the aa^1 side chain. Preferred side chains comprise, or consist of, methyl substituted by one or two 5- or 6- membered rings.

More preferably, aa^1 is Phe, Dpa or a wholly or partially hydrogenated analogue thereof. The wholly hydrogenated analogues are Cha and D-Dcha.

aa^2 is an imino acid having from 4 to 6 ring members.

A preferred class of products comprises those in which aa^2 is a residue of an imino acid of formula (IV)



(IV),

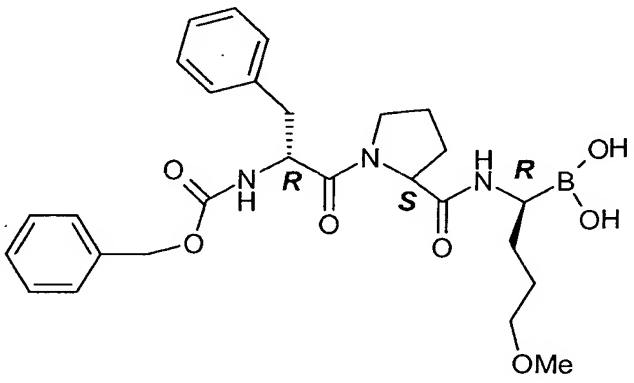
where R^{11} is $-CH_2-$, CH_2-CH_2-, $-S-CH_2-$ or $-CH_2-CH_2-CH_2-$, which group when the ring is 5 or 6-membered is optionally substituted at one or more $-CH_2-$ groups by from 1 to 3 C_1-C_3 alkyl groups, for example to form the R^{11} group $-S-C(CH_3)_2-$. Of these imino acids, azetidine-2-carboxylic acid, especially (s)-azetidine-2-carboxylic acid, and more particularly proline are preferred.

It will be appreciated from the above that a very preferred class of products consists of those in which aa^1-aa^2 is Phe-Pro. In another preferred class, aa^1-aa^2 is Dpa-Pro. In other products, aa^1-aa^2 is Cha-Pro or Dcha-Pro. Of course, the invention includes corresponding product classes in which Pro is replaced by (s)-azetidine-2-carboxylic acid.

R^9 is as defined previously and may be a moiety R^1 of the formula $-(CH_2)_s-Z$. Integer s is 2, 3 or 4 and W is –OH, –OMe, –OEt or halogen (F, Cl, I or, preferably, Br). The most preferred Z groups are –OMe and –OEt, especially –OMe. It is preferred that s is 3 for all Z groups and, indeed, for all compounds of the invention. Particularly preferred R^1 groups are 2-bromoethyl, 2-chloroethyl, 2-methoxyethyl, 4-bromobutyl, 4-chlorobutyl, 4-methoxybutyl and, especially, 3-bromopropyl, 3-chloropropyl and 3-methoxypropyl. Most preferably, R^1 is 3-methoxypropyl. 2-Ethoxyethyl is another preferred R^1 group.

Accordingly, a very preferred class of salts consists of those of acids of the formula X-Phe-Pro-Mpg-$B(OH)_2$, especially Cbz-Phe-Pro-Mpg-$B(OH)_2$; also preferred are analogues of these compounds in which Mpg is replaced by a residue with another of the particularly preferred R^1 groups and/or Phe is replaced by Dpa or another aa^1 residue.

The aa^1 moiety of the salt is preferably of R configuration (D-configuration). The aa^2 moiety is preferably of S configuration (L-configuration). Particularly preferred salts have aa^1 of R configuration and aa^2 of S configuration. The chiral centre $-NH-CH(R^1)-B-$ is preferably of R configuration. It is considered that commercial formulations will have the chiral centres in RSR arrangement, as for example in the case of salts of Cbz-Phe-Pro-BoroMpg-OH:



Cbz-(R)-Phe-(S)-Pro-(R)-boroMpg-OH

The invention includes salts of Cbz-(R)-Phe-(S)-Pro-(R)-boroMpg-OH (and of other compounds of the formula X-(R)-Phe-(S)-Pro-(R)-boroMpg-OH) which are at least 90% pure, e.g. at least 95% pure, for example purities of up to 99% or exceeding 99%, e.g. up to 99.5%.

In broad terms, the salts described herein may be considered to correspond to reaction products of an organoboronic acid as described above with a strong base, e.g. a basic metal compound; the salts are however not limited to products resulting from such a reaction and may be obtained by alternative routes.

The salts are therefore obtainable by contacting an acid of formula (I) with a strong base. The invention thus contemplates products (compositions of matter) having the characteristics of a reaction product of an acid of formula (I) and a strong base. The base is pharmaceutically acceptable.

As suitable salts may be mentioned:

1. Salts of metals, notably monovalent metals, as which may be mentioned alkali metals;

2. Salts of strongly basic organic nitrogen-containing compounds, including:

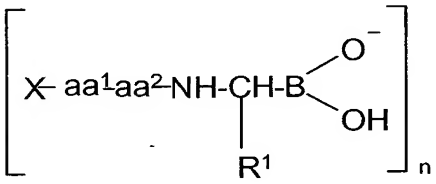
2A. Salts of guanidines and their analogues;

2B. Salts of strongly basic amine, examples of which include (i) aminosugars and (ii) other amines.

Of the above salts, the most preferred are alkali metals, especially Na and Li, and aminosugars.

Preferred salts are of the acid boronate though in practice the acid salts may contain a very small proportion of the doubly deportonated boronate. The term "acid boronate" refers to trigonal $-B(OH)_2$ groups in which one of the B-OH groups is deprotonated as well as to corresponding tetrahedral groups in equilibrium therewith. Acid boronates have a stoichiometry consistent with single deprotonation.

The invention includes therefore products (compositions of matter) which comprise salts of formula (V):



(V)

where Y^{n+} is a pharmaceutically acceptable cation obtainable from a strong base, and aa^1, aa^2, X and R^1 are as defined above. Also included are products in which R^1 is replaced by another R^9 group.

The salts preferably have a solubility of at least 10 mM, more preferably at least 20mM, when their solubility is determined as described in the examples at a dissolution of 25mg/ml. More preferably yet they have a solubility of least 50mM when their solubility is determined as described in the examples at a dissolution of 50mg/ml.

The invention includes salts of boronic acids (I) having an observed stoichiometry consistent with the salt being of (being representable by) the formula "$(boronate)_n$ $cation^{n+}$". One class of such salts are represented by the formula:

$$[Cbz\text{-}(R)\text{-}Phe\text{-}(S)\text{-}Pro\text{-}(R)\text{-}Mpg\text{-}B(OH)(O^-)]M^+$$

where M^+ represents a monovalent cation, especially an alkali metal cation. It will be understood that the above representation is a notional representation of a product whose observed stoichiometry is unlikely to be literally and exactly 1:1. In the above formula, the trigonally-represented boronate represents, as always, boronates which are trigonal, tetrahedral or mixed trigonal/tetrahedral.

Particularly preferred are products which comprise:

(i) species selected from (a) acids of formula (VIII): X-(R)-Phe-(S)-Pro-(R)-Mpg-$B(OH)_2$ where X is H or an amino-protecting group, especially Cbz, and (b) boronate anions thereof; and

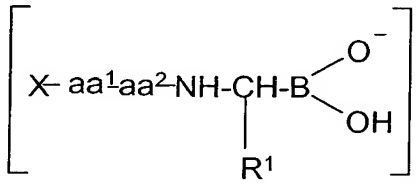
(ii) ions having a valency V in combination with said species, the species and said ions having an observed stoichiometry consistent with a notional species:ion stoichiometry of V:1. In one class of salts, V is 1.

Considering the counter-ions in turn:

1. Monovalent metal, especially alkali metal salts

Suitable alkali metals include lithium, sodium and potassium. All of these are remarkably soluble. Lithium and sodium are particularly preferred because of their high solubility. The lithium and particularly sodium salts are of surprisingly high solubility in relation to potassium amongst others. Sodium is most preferred. Salts containing mixtures of alkali metals are contemplated by the invention.

The invention includes products comprising salts of the formula (VI)



(VI)

where M^+ is an alkali metal ion and aa^1, aa^2, X and R^1 are as defined above, as well as salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical M^+ group) and mixtures of such salts. Included also are products wherein R^1 is replaced by another R^9 group.

2. Strongly basic organic nitrogen-containing compounds

The invention includes products obtainable by (having the characteristics of a product obtained by) reaction of a peptide boronic acid as defined above and a strong organic base. Two preferred classes of organic base are described in sections 2A and 2B below. Particularly preferred are acid salts (in which one of the two boronic –OH groups is deprotonated). Most commonly, the salts contain a single type of organic counter-ion (disregarding trace contaminants) but the invention contemplates salts containing mixtures of organic counter-ions; in one sub-class, the different counter-ions all fall within the section 2A family described below or, as the case may be, in the

section 2B family below; in another subclass, the salts comprise a mixture of organic counter-ions which are not all from the same family (2A or 2B).

Suitable organic bases include those with a pkb of 7 or more, e.g. 7.5 or more, for example in the region of 8 or more. Bases which are less lipophilic [e.g. have at least one polar functional group (e.g. 1, 2 or 3 such groups) for example hydroxy] are favoured; thus aminosugars are one favoured class of base.

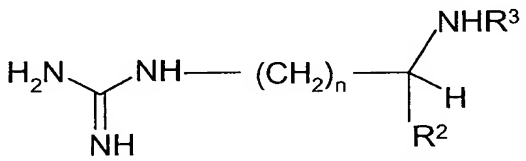
2A. Guanidines and their analogues

The guanidino compound (guanidine) may in principle be any soluble and pharmaceutically acceptable compound having a guanidino or a substituted guanidino group, or a substituted or unsubstituted guanidine analogue. Suitable substituents include aryl (e.g. phenyl), alkyl or alkyl interrupted by an ether or thioether linkage and, in any event, typically contain from 1 to 6 and especially 1, 2, 3, or 4 carbon atoms, as in the case of methyl or ethyl. The guanidino group may have 1, 2, 3 or 4 substituent groups but more usually has 1 or 2 substituent groups, preferably on a terminal nitrogen. One class of preferred guanidine is monoalkylated; another class is dialkylated. As guanidine analogues may be mentioned thioguanidines and 2-amino pyridines. Compounds having unsubstituted guanidino groups, for example guanidine and arginine, form one particularly preferred class.

Salts containing mixtures of guanidines are contemplated by the invention.

The guanidino compound is preferably L-arginine or an L-arginine analogue, for example D-arginine, or the D- or, preferably, L- isomers of homoarginine or agmatine [(4-aminobutyl) guanidine]. Less preferred arginine analogues are NG-nitro-L-arginine methyl ester, for example, and constrained guanidine analogues, particularly 2-amino pyrimidines, for example 2,6-quinazolinediamines such as 5,6,7,8-tetrahydro-2,6-quinazolinediamine, for example. The guanidino compound may also be a peptide, for example a dipeptide, containing arginine; one such dipeptide is L-tyrosyl-L-arginine.

Some particularly preferred guanidino compounds are compounds of formula (VII):

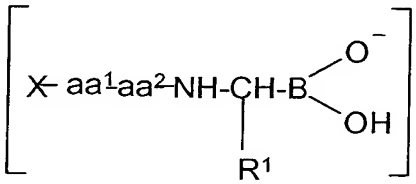


(VII)

where n is from 1 to 6 and preferably at least 2, e.g. 3 or more, and preferably no more than 5. Most preferably, n is 3, 4 or 5. R^2 is H or carboxylate or derivatised carboxylate, for example to form an ester (e.g. a C_1-C_4 alkyl ester) or amide. R^3 is H, C_1-C_4 alkyl or a residue of a natural or unnatural amino acid (e.g. tyrosine). The compounds of formula (IV) are usually of L-configuration. The

compounds of formula (IV) are arginine (n=3; R^2=carboxyl; R^3=H) and arginine derivatives or analogues.

The invention includes products comprising salts of the formula (IX)



(IX)

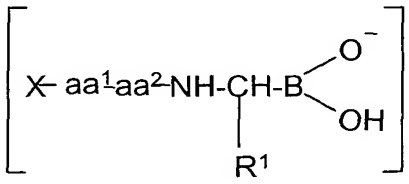
where aa^1, aa^2, X and R^1 are as defined previously and G^+ is the protonated form of a pharmaceutically acceptable organic compound comprising a guanidino group or an analogue thereof, as well as salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical G^+ group) and mixtures of such salts. Also included are products wherein R^1 is replaced by another R^9 group.

2B. Strongly basic amines

The invention includes products obtainable by (having the characteristics of a product obtained by) reaction of a peptide boronic acid as defined above and a strong organic base which is an amine. The amine may in principle be any soluble and pharmaceutically acceptable amine.

It is envisaged that a desirable class of amine includes those having polar functional groups in addition to a single amine group, as such compounds will be more hydrophilic and thus more soluble than others. Preferably, the or each additional functional group is hydroxy. Some amines have 1, 2, 3, 4, 5 or 6 additional functional groups, especially hydroxy groups. In one particularly preferred class of amines the ratio of (amino plus hydroxy groups):carbon atoms is from 1:2 to 1:1, the latter ratio being particularly preferred. These amines with one or more additional polar functional groups may be a hydrocarbon, especially an alkane, substituted by the amino group and the additional polar group(s). The amino group may be substituted or unsubstituted and, excluding amino substituents, the polar base may contain, for example, up to 10 carbon atoms; usually there are no less than three such carbon atoms, e.g. 4, 5 or 6. Aminosugars are included in this category of polar bases.

The invention includes products comprising salts of the formula (X)



(X)

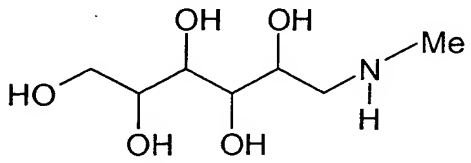
where aa^1, aa^2, X and R^1 are as defined previously and A^+ is the protonated form of a pharmaceutically acceptable amine, as well as salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical A^+ group) and mixtures of such salts. In one class of such products, A^+ is the protonated form of an amine described in section 2B(i) below; in another class A^+ is the protonated form of an amine described in 2B(ii) below. Also included are products in which R^1 is replaced by another R^9 group.

Two preferred classes of amine base are described in sections 2B(i) and 2B(ii) below. Particularly preferred are acid salts (in which one of the two boronic –OH groups is deprotonated). Most commonly, the salts contain a single type of amine counter-ion (disregarding trace contaminants) but the invention contemplates salts containing mixtures of amine counter-ions; in one sub-class, the different counter-ions all fall within the sub-section 2B(i) family described below or, as the case may be, in the sub-section 2B(ii) family below; in another subclass, the salts comprise a mixture of organic counter-ions which are not all from the same family (2B(i) or 2B(ii)).

2B(i) Aminosugars

The identity of the aminosugar is not critical to the invention. Preferred aminosugars include ring-opened sugars, especially glucamines. Cyclic aminosugars are also envisaged as useful. One class of the aminosugars is N-unsubstituted and another, preferred, class is N-substituted by one or two N-substituents (preferably one). Suitable substituents are hydrocarbyl groups, for example and without limitation containing from 1 to 12 carbon atoms; the substituents may comprise alkyl or aryl moieties or both. Preferred substituents are C_1, C_2, C_3, C_4, C_5, C_6, C_7 and C_8 alkyl groups, in particular methyl and ethyl, of which methyl is most preferred. Data indicate that aminosugars, especially N-methyl-D-glucamine, are of surprisingly high solubility.

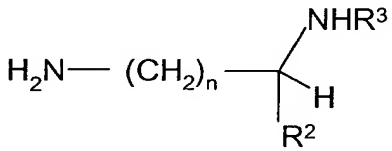
A most preferred aminosugar is N-methyl-D-glucamine:



2B(ii) Other amines

Other suitable amines include amino acids (whether naturally occurring or not) whose side chain is substituted by an amino group, especially lysine.

Some amines are compounds of formula (XI):



(XI)

where n, R^2 and R^3 are as defined in relation to formula (IV). The compounds of formula (VI) are usually of L-configuration. The compounds of formula (VI) are lysine (n=4; R^2=carboxyl; R^3=H) and lysine derivatives or analogues. A most preferred amine is L-lysine.

Other suitable amines are nitrogen-containing heterocycles. At least usually, such heterocyclic compounds are alicyclic; one class of the heterocyclic compounds is N-substituted and another, preferred, class is N-unsubstituted. The heterocycles may contain 6 ring-forming atoms, as in the cases of piperidine, piperazine and morpholine. One class of amines includes N-containing heterocycles substituted by polar substituents, especially hydroxy, e.g. 1, 2 or 3 times.

The invention therefore includes amines other than aminosugars which have one or more (e.g. 1, 2, 3, 4, 5 or 6) polar substituents, especially hydroxy, in addition to one amine group. Such compounds may have a ratio of (amino plus hydroxy groups):carbon atoms of 1:2 to 1:1, the latter ratio being particularly preferred.

The invention includes mixed salts, i.e. salts containing a mixture of boropeptide moieties and/or counterions but single salts are preferred.

The salts in solid form may contain a solvent, e.g. water.

Use of the Products of the Invention

The salts of the invention are thrombin inhibitors. They are therefore useful for inhibiting thrombin. The invention therefore provides compounds which have potential for controlling haemostasis and

especially for inhibiting coagulation, for example preventing secondary events after myocardial infarction. The medical use of the compounds may be prophylactic (including to prevent occurrence of thrombosis) as well as therapeutic (including to prevent re-occurrence of thrombosis or secondary thrombotic events).

The salts may be employed when an anti-thrombogenic agent is needed. They are thus indicated in the treatment or prophylaxis of thrombosis and hypercoagulability in blood and tissues of animals including man. The term "thrombosis" includes *inter alia* atrophic thrombosis, arterial thrombosis, cardiac thrombosis, coronary thrombosis, creeping thrombosis, infective thrombosis, mesenteric thrombosis, placental thrombosis, propagating thrombosis, traumatic thrombosis and venous thrombosis.

It is known that hypercoagulability may lead to thromboembolic diseases.

Examples of venous thromboembolism which may be treated or prevented with compounds of the invention include obstruction of a vein, obstruction of a lung artery (pulmonary embolism), deep vein thrombosis, thrombosis associated with cancer and cancer chemotherapy, thrombosis inherited with thrombophilic diseases such as Protein C deficiency, Protein S deficiency, antithrombin III deficiency, and Factor V Leiden, and thrombosis resulting from acquired thrombophilic disorders such as systemic lupus erythematosus (inflammatory connective tissue disease). Also with regard to venous thromboembolism, compounds of the invention are useful for maintaining patency of indwelling catheters.

Examples of cardiogenic thromboembolism which may be treated or prevented with compounds of the invention include thromboembolic stroke (detached thrombus causing neurological affliction related to impaired cerebral blood supply), cardiogenic thromboembolism associated with atrial fibrillation (rapid, irregular twitching of upper heart chamber muscular fibrils), cardiogenic thromboembolism associated with prosthetic heart valves such as mechanical heart valves, and cardiogenic thromboembolism associated with heart disease.

Examples of arterial thrombosis include unstable angina (severe constrictive pain in chest of coronary origin), myocardial infarction (heart muscle cell death resulting from insufficient blood supply), ischemic heart disease (local anemia due to obstruction (such as by arterial narrowing) of blood supply), reocclusion during or after percutaneous transluminal coronary angioplasty, restenosis after percutaneous transluminal coronary angioplasty, occlusion of coronary artery bypass grafts, and occlusive cerebrovascular disease. Also with regard to arterial thrombosis, anti-thrombotic compounds of the invention are useful for maintaining patency in arteriovenous cannulas.

Other conditions associated with hypercoagulability and thromboembolic diseases which may be mentioned inherited or acquired deficiencies in heparin cofactor II, circulating antiphospholipid antibodies (Lupus anticoagulant), homocysteinemi, heparin induced thrombocytopenia and defects in fibrinolysis.

Particular uses which may be mentioned include the therapeutic and/or prophylactic treatment of venous thrombosis and pulmonary embolism. Preferred indications envisaged for the products of the invention (notably the salts of TRI 50c) include:

- Prevention of venous thromboembolic events (e.g. deep vein thrombosis and/or pulmonary embolism). Examples include patients undergoing orthopaedic surgery such as total hip replacement, total knee replacement, major hip or knee surgery; patients undergoing general surgery at high risk for thrombosis, such as abdominal or pelvic surgery for cancer; and in patients bedridden for more than 3 days and with acute cardiac failure, acute respiratory failure, infection.
- Prevention of thrombosis in the haemodialysis circuit in patients, particularly patients with end stage renal disease.
- Prevention of cardiovascular events (death, myocardial infarction, etc) in patients with end stage renal disease, whether or not requiring haemodialysis sessions.
- Prevention of venous thrombo-embolic events in patients receiving chemotherapy through an indwelling catheter.
- Prevention of thromboembolic events in patients undergoing lower limb arterial reconstructive procedures (bypass, endarteriectomy, transluminal angioplasty, etc).
- Treatment of venous thromboembolic events.
- Prevention of cardiovascular events in acute coronary syndromes (e.g. unstable angina, non Q wave myocardial ischaemia/infarction), in combination with another cardiovascular agent, for example aspirin (acetylsalicylic acid; aspirin is a registered trade mark in Germany), thrombolytics (see below for examples), antiplatelet agents (see below for examples).
- Treatment of patients with acute myocardial infarction in combination with acetylsalicylic acid, thrombolytics (see below for examples).

The thrombin inhibitors of the invention are thus indicated both in the therapeutic and/or prophylactic treatment of all the aforesaid disorders.

In one method, the products of the invention are used for the treatment of patients by dialysis, by providing the product in the dialysis solution, as described in relation to other thrombin inhibitors in WO 00/41715, which is incorporated herein by reference. The invention therefore includes dialysing solutions and dialysing concentrates which comprise a product of the invention, as well as a method of treatment by dialysis of a patient in need of such treatment, which method comprises the use of a dialysing solution including a low molecular weight thrombin inhibitor. Also included is the use of an

anti-thrombotic product of the invention for the manufacture of a medicament for the treatment by dialysis of a patient, in which the anti-thrombotic product of the invention is provided in the dialysing solution.

In another method, the products of the invention are used to combat undesirable cell proliferation, as described in relation to other thrombin inhibitors in WO 01/41796, which is incorporated herein by reference. The undesirable cell proliferation is typically undesirable hyperplastic cell proliferation, for example proliferation of smooth muscle cells, especially vascular smooth muscle cells. The products of the invention particularly find application in the treatment of intimal hyperplasia, one component of which is proliferation of smooth muscle cells. Restenosis can be considered to be due to neointimal hyperplasia; accordingly intimal hyperplasia in the context of the invention includes restenosis.

The products of the invention are also contemplated for the treatment of ischemic disorders. More particularly, they may be used in the treatment (whether therapeutic or prophylactic) of an ischemic disorder in a patient having, or at risk of, non-valvular atrial fibrillation (NVAF) as described in relation to other thrombin inhibitors in WO 02/36157, which is incorporated herein by reference. Ischemic disorders are conditions whose results include a restriction in blood flow to a part of the body. The term will be understood to include thrombosis and hypercoagulability in blood, tissues and/or organs. Particular uses that may be mentioned include the prevention and/or treatment of ischemic heart disease, myocardial infarction, systemic embolic events in e.g. the kidneys or spleen, and more particularly of cerebral ischemia, including cerebral thrombosis, cerebral embolism and/or cerebral ischemia associated with non-cerebral thrombosis or embolism (in other words the treatment (whether therapeutic or prophylactic) of thrombotic or ischemic stroke and of transient ischemic attack), particularly in patients with, or at risk of, NVAF.

The products of the invention are also contemplated for the treatment of rheumatic/arthritic disorders, as described in relation to other thrombin inhibitors in WO 03/007984, which is incorporated herein by reference. Thus, the products of the invention may be used in the treatment of chronic arthritis, rheumatoid arthritis, osteoarthritis or ankylosing spondylitis

Moreover, the products of the invention are expected to have utility in prophylaxis of re-occlusion (i.e. thrombosis) after thrombolysis, percutaneous trans-luminal angioplasty (PTA) and coronary bypass operations; the prevention of re-thrombosis after microsurgery and vascular surgery in general. Further indications include the therapeutic and/or prophylactic treatment of disseminated intravascular coagulation caused by bacteria, multiple trauma, intoxication or any other mechanism; anticoagulant treatment when blood is in contact with foreign surfaces in the body such as vascular grafts, vascular stents, vascular catheters, mechanical and biological prosthetic valves or any other

medical device; and anticoagulant treatment when blood is in contact with medical devices outside the body such as during cardiovascular surgery using a heart-lung machine or in haemodialysis.

The products of the invention are further indicated in the treatment of conditions where there is an undesirable excess of thrombin without signs of hypercoagulability, for example in neurodegenerative diseases such as Alzheimer's disease. In addition to its effects on the coagulation process, thrombin is known to activate a large number of cells (such as neutrophils, fibroblasts, endothelial cells and smooth muscle cells). Therefore, the compounds of the invention may also be useful for the therapeutic and/or prophylactic treatment of idiopathic and adult respiratory distress syndrome, pulmonary fibrosis following treatment with radiation or chemotherapy, septic shock, septicaemia, inflammatory responses, which include, but are not limited to, edema, acute or chronic atherosclerosis such as coronary arterial disease, cerebral arterial disease, peripheral arterial disease, reperfusion damage, and restenosis after percutaneous trans-luminal angioplasty (PTA).

The salts may also be useful in the treatment of pancreatitis.

The salts described herein are further considered to be useful for inhibiting platelet procoagulant activity. The invention provides a method for inhibiting platelet pro-coagulant activity by administering a salt of a boronic acid described herein to a mammal at risk of, or suffering from, arterial thrombosis, particularly a human patient. Also provided is the use of such salts for the manufacture of medicaments for inhibiting platelet procoagulant activity.

The use of products of the invention as inhibitors of platelet pro-coagulant activity is predicated on the observation that the boronic acids described herein are indicated to be effective at inhibiting arterial thrombosis as well as venous thrombosis.

Indications involving arterial thrombosis include acute coronary syndromes (especially myocardial infarction and unstable angina), cerebrovascular thrombosis and peripheral arterial occlusion and arterial thrombosis occurring as a result of atrial fibrillation, valvular heart disease, arterio-venous shunts, indwelling catheters or coronary stents. Accordingly, in another aspect the invention provides a method of treating a disease or condition selected from this group of indications, comprising administering to a mammal, especially a human patient, a salt of the invention. The invention includes products for use in an arterial environment, e.g. a coronary stent or other arterial implant, having a coating which comprises a salt of the invention.

The salts of the invention may be used prophylactically to treat an individual believed to be at risk of suffering from arterial thrombosis or a condition or disease involving arterial thrombosis or therapeutically (including to prevent re-occurrence of thrombosis or secondary thrombotic events).

The invention therefore includes the use of selective thrombin inhibitors (organoboronic acid salts) described herein for treatment of the above disorders by prophylaxis or therapy as well as their use in pharmaceutical formulations and the manufacture of pharmaceutical formulations.

Administration and Pharmaceutical Formulations

The salts may be administered to a host, for example, in the case where the drug has anti-thrombogenic activity, to obtain an anti-thrombogenic effect. In the case of larger animals, such as humans, the compounds may be administered alone or in combination with pharmaceutically acceptable diluents, excipients or carriers. The term "pharmaceutically acceptable" includes acceptability for both human and veterinary purposes, of which acceptability for human pharmaceutical use is preferred. In the case of oral administration, the compounds are preferably administered in a form which prevents the salt of the invention from contact with the acidic gastric juice, such as enterically coated formulations, which thus prevent release of the salt of the invention until it reaches the duodenum.

The enteric coating is suitably made of carbohydrate polymers or polyvinyl polymers, for example. Examples of enteric coating materials include, but are not limited to, cellulose acetate phthalate, cellulose acetate succinate, cellulose hydrogen phthalate, cellulose acetate trimellitate, ethyl cellulose, hydroxypropyl-methylcellulose phthalate, hydroxypropylmethylcellulose acetate succinate, carboxymethyl ethylcellulose, starch acetate phthalate, amylose acetate phthalate, polyvinyl acetate phthalate, polyvinyl butyrate phthalate, styrene-maleic acid copolymer, methyl-acrylate-methacrylic acid copolymer (MPM-05), methylacrylate-methacrylic acid-methylmethacrylate copolymer (MPM-06), and methylmethacrylate-methacrylic acid co-polymer (Eudragit® L & S). Optionally, the enteric coating contains a plasticiser. Examples of the plasticiser include, but are not limited to, triethyl citrate, triacetin, and diethyl phthalate.

The salts of the invention may be combined and/or co-administered with any cardiovascular treatment agent. There are large numbers of cardiovascular treatment agents available in commercial use, in clinical evaluation and in pre-clinical development, which could be selected for use with a product of the invention for the prevention of cardiovascular disorders by combination drug therapy. Such agent can be one or more agents selected from, but not limited to several major categories, namely, a lipid-lowering drug, including an IBAT inhibitor, a fibrate, niacin, a statin, a CETP inhibitor, and a bile acid sequestrant, an anti-oxidant, including vitamin E and probucol, a IIb/IIIa antagonist (e.g. xemilofiban and orbofiban), an aldosterone inhibitor (e.g. spirolactone and epoxymexrenone), an adenosine A2 receptor antagonist (e.g. losartan), an adenosine A3 receptor agonist, a beta-blocker, acetylsalicylic acid, a loop diuretic and an ace inhibitor.

The salts of the invention may be combined and/or co-administered with any antithrombotic agent with a different mechanism of action, such as the antiplatelet agents acetylsalicylic acid, ticlopidine, clopidogrel, thromboxane receptor and/or synthetase inhibitors, fibrinogen receptor antagonists, prostacyclin mimetics and phosphodiesterase inhibitors and ADP-receptor (P_2 T) antagonists.

The products of the invention may further be combined and/or co-administered with thrombolytics such as tissue plasminogen activator (natural, recombinant or modified), streptokinase, urokinase, prourokinase, anisoylated plasminogen-streptokinase activator complex (APSAC), animal salivary gland plasminogen activators, and the like, in the treatment of thrombotic diseases, in particular myocardial infarction.

The products of the invention may be combined and/or co-administered with antiplatelet agents, e.g. ticlopidine, clopidogrel, abciximab, eptifibatide, tirofiban.

The salts of the invention may be combined and/or co-administered with a cardioprotectant, for example an adenosine A1 or A3 receptor agonist.

There is also provided a method for treating an inflammatory disease in a patient that comprises treating the patient with a product of the invention and an NSAID, e.g., a COX-2 inhibitor. Such diseases include but are not limited to nephritis, systemic lupus, erythematosus, rheumatoid arthritis, glomerulonephritis, vasculitis and sacoidosis. Accordingly, the anti-thrombotic salts of the invention may be combined and/or co-administered with an NSAID.

Typically, therefore, the salts described herein may be administered to a host to obtain a thrombin-inhibitory effect, or in any other thrombin-inhibitory or anti-thrombotic context mentioned herein.

Actual dosage levels of active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular patient, compositions, and mode of administration. The selected dosage level will depend upon the activity of the particular compound, the severity of the condition being treated and the condition and prior medical history of the patient being treated. However, it is within the skill of the art to start doses of the compound at levels lower than required for to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

For example, it is currently contemplated that, in the case of oral administration of salts of TRI 50c, the salts might for instance be administered in an amount of from 0.5 to 2.5mg/Kg twice daily, calculated as TRI 50c. Other salts might be administered in equivalent molar amounts. The

invention is not limited to administration in such quantities or regimens and includes dosages and regimens outside those described in the previous sentence.

According to a further aspect of the invention there is provided an oral pharmaceutical formulation including a product of the invention, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

Solid dosage forms for oral administration include capsules, tablets (also called pills), powders and granules. In such solid dosage forms, the active compound is typically mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or one or more: a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol and silicic acid; b) binders such as carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose and acacia; c) humectants such as glycerol; d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates and sodium carbonate; e) solution retarding agents such as paraffin; f) absorption accelerators such as quaternary ammonium compounds; g) wetting agents such as cetyl alcohol and glycerol monostearate; h) absorbents such as kaolin and bentonite clay and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate and mixtures thereof. In the case of capsules and tablets, the dosage form may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycol, for example.

Suitably, the oral formulations may contain a dissolution aid. The dissolution aid is not limited as to its identity so long as it is pharmaceutically acceptable. Examples include nonionic surface active agents, such as sucrose fatty acid esters, glycerol fatty acid esters, sorbitan fatty acid esters (e.g., sorbitan trioleate), polyethylene glycol, polyoxyethylene hydrogenated castor oil, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene alkyl ethers, methoxypolyoxyethylene alkyl ethers, polyoxyethylene alkylphenyl ethers, polyethylene glycol fatty acid esters, polyoxyethylene alkylamines, polyoxyethylene alkyl thioethers, polyoxyethylene polyoxypropylene copolymers, polyoxyethylene glycerol fatty acid esters, pentaerythritol fatty acid esters, propylene glycol monofatty acid esters, polyoxyethylene propylene glycol monofatty acid esters, polyoxyethylene sorbitol fatty acid esters, fatty acid alkylolamides, and alkylamine oxides; bile acid and salts thereof (e.g., chenodeoxycholic acid, cholic acid, deoxycholic acid, dehydrocholic acid and salts thereof, and glycine or taurine conjugate thereof); ionic surface active agents, such as sodium laurylsulfate, fatty acid soaps, alkylsulfonates, alkylphosphates, ether phosphates, fatty acid salts of basic amino acids; triethanolamine soap, and alkyl quaternary ammonium salts; and amphoteric surface active agents, such as betaines and aminocarboxylic acid salts.

The active compounds may also be in micro- encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan and mixtures thereof. Besides inert diluents, the oral compositions may also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavouring and perfuming agents. Suspensions, in addition to the active compounds, may contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminium metahydroxide, bentonite, agar-agar, and tragacanth and mixtures thereof.

The product of the invention may be presented as solids in finely divided solid form, for example they may be micronised. Powders or finely divided solids may be encapsulated.

The active compound may be given as a single dose, in multiple doses or as a sustained release formulation.

Synthesis

1. Peptide/Peptidomimetic Synthesis

The synthesis of boropeptides, including, for example, Cbz-D-Phe-Pro-BoroMpg-OPinacol is familiar to those skilled in the art and described in the prior art mentioned above, including Claeson et al (US 5574014 and others) and Kakkar et al (WO 92/07869 and family members including US 5648338). It is described also by Elgendy et al *Adv. Exp. Med. Biol. (USA)* **1993**, *340*, 173-178; Claeson,G. et al *Biochem.J.* **1993**, *290*, 309-312; Deadman et al *J. Enzyme Inhibition* **1995**, *9*, 29-41, and by Deadman et al *J. Med. Chem.* **1995**, *38*, 1511-1522.

Stereoselective synthesis with S or R configuration at the chiral B-terminal carbon may be conducted using established methodology (Elgendy et al *Tetrahedron. Lett.* **1992**, *33*, 4209-4212; WO 92/07869 and family members including US 5648338) using (+) or (—)- pinanediol as the chiral director (Matteson et al *J. Am. Chem. Soc.* **1986**, *108*, 810-819; Matteson et al *Organometallics..* **1984**, *3*, 1284-1288). Another approach is to resolve the requisite aminoboronate intermediate (e.g. Mpg-BOPinacol) to selectively obtain the desired (R)-isomer and couple it to the dipeptide

moiety (e.g. Cbz-(R)-Phe-(S)-Pro, which is the same as Cbz-D-Phe-L-Pro) which will form the remainder of the molecule.

The boropeptides may be synthesised initially in the form of boronic acid esters, particularly esters with diols. Such diol esters may be converted to the peptide boronic acid as described next.

2. Ester to Acid Conversion

A peptide boronate ester such as Cbz-(R)-Phe-Pro-BoroMpg-OPinacol may be hydrolysed to form the corresponding acid, for example as described in Example 1 below, Section H.

A novel technique for converting a diol ester of a peptide boronic acid of formula (I) into the acid comprises dissolving the diol ester in an ether and particularly a dialkyl ether, reacting the thus-dissolved diol with a diolamine, for example a dialkanolamine, to form a product precipitate, recovering the precipitate, dissolving it in a polar organic solvent and reacting the thus-dissolved product with an aqueous medium, e.g. an aqueous acid, to form the peptide boronic acid. The boronic acid may be recovered from the organic layer of the mixture resulting from the reaction, for example by removing the solvent, e.g. by evaporation under vacuum or distillation. The reaction between the diol ester and the diolamine may be carried out under reflux, for example.

The identity of the diol is not critical to the invention. As suitable diols may be mentioned aliphatic and aromatic compounds having hydroxy groups that are substituted on adjacent carbon atoms or on carbon atoms substituted by another carbon. That is to say, suitable diols include compounds having at least two hydroxy groups separated by at least two connecting carbon atoms in a chain or ring. One class of diols comprises hydrocarbons substituted by exactly two hydroxy groups. One such diol is pinacol and another is pinanediol; there may also be mentioned neopentylglycol, 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 2,3-butanediol, 1,2-diisopropylethanediol, 5,6-decanediol and 1,2-dicyclohexylethanediol.

The alkyl groups of the dialkyl ether preferably have 1, 2, 3 or 4 carbon atoms and the alkyl groups may be the same or different. A most preferred ether is diethyl ether.

The alkyl groups of the dialkanolamine preferably have 1, 2, 3 or 4 carbon atoms and the alkyl groups may be the same or different. A most preferred dialkanolamine is diethanolamine. The diethanolamine/boronic acid reaction product hydrolyses in water at room temperature and the rate of hydrolysis may be accelerated by adding acid or base.

The polar organic solvent is preferably $CHCl_3$. Other examples are polyhalogenated alkanes generally and ethyl acetate. In principle, any polar organic solvent is acceptable other than alcohols.

The aqueous acid is suitably a strong inorganic acid at a pH in the region of 1; hydrochloric acid is most preferred.

After reaction with the acid, the reaction mixture is suitably washed with, for example, NH_4Cl or another mild base.

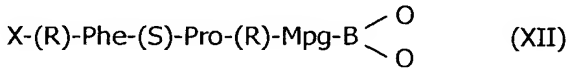
A preferred procedure is as follows

1. The pinacol or pinanediol ester of the selected peptide boronic acid is dissolved in diethylether.
2. Diethanolamine is added and the mixture is refluxed at 40 °C.
3. The precipitated product is removed (filtered), washed (usually several times) with diethyl ether or another polar organic solvent other than an alcohol, and dried (e.g. by evaporation under vacuum).
4. The dry product is dissolved in a polar organic solvent other than an alcohol, e.g. $CHCl_3$. Aqueous acid or base is added ,e.g hydrochloric acid (pH 1), and the mixture is stirred for e.g. approximately 1h at room temperature.
5. The organic layer is removed and washed with NH_4Cl solution.
6. The organic solvent is distilled off and the residual solid product is dried.

The above process results in the formation of what may conveniently be referred to as a "diolamine adduct" of the peptide boronic acids of formula (I), especially such adducts with diethanolamine, and such adducts are themselves included in the invention. The molecular structure of such adducts is not known: they might comprise a compound in which the two oxygens and the nitrogen of the diolamine are all coordinated to the boron; they might comprise ions. A particular novel product included in the invention is that obtainable by reacting a pinacol or pinanediol ester of a compound of Formula VIII, particularly (RSR)-TRI 50c, and diethanolamine, i.e. the novel product is an (RSR)-TRI 50c/diethanolamine "adduct" where the acid is (RSR)-TRI 50c.

The diolamine materials of the invention may be defined as a composition of matter comprising:

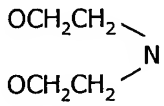
(i) a species of formula (XII)



(XII)

wherein X is H or an amino protecting group, the boron atom is optionally coordinated additionally with a nitrogen atom, and the valency status of the terminal oxygens is open (they may be attached to a second covalent bond, be ionised as $-O^-$, or have some other, for example intermediate, status); and, in bonding association therewith

(ii) a species of formula (XIII)



(XIII)

wherein the valency status of the nitrogen atom and the two oxygen atoms is open. It will be appreciated that the terminal oxygen atoms of the species of formula (IX) and the oxygen atoms of the species of formula (X) may be the same oxygen atoms, in which case the species of formula (X) forms a diol ester with the species of formula (IX).

It will be appreciated that the aforegoing technique comprises an example of a method for recovering an organoboronic acid product, the method comprising providing in a solvent a dissolved mixture comprising the organoboronic acid in a soluble form and a compound having two hydroxy groups and an amino group (i.e. a diolamine), causing or allowing the organoboronic acid and the diolamine to react to form a precipitate, and recovering the precipitate. The soluble form of the organoboronic acid may be a diol ester, as discussed above. The solvent may be an ether, as discussed above. The organoboronic acid may be one of the organoboronic acids referred to in this specification, for example it may be of Formula (I), (II) or (III). The method described in this paragraph is novel and forms an aspect of the invention. A recovery method is filtration.

The reaction between the diolamine and the soluble form of the organoboronic acid is suitable carried out at an elevated temperature, for example under reflux.

Another aspect of the invention is a method for recovering an organoboron species, comprising

providing, in a form soluble in an ether, an organoboronic acid, for example a drug such as, e.g. , a compound of formula (III);

forming a solution of the soluble form in the ether;

combining the solution with a dialkanolamine and allowing or causing the dialkanolamine to react with the soluble form of the organoboronic acid to form an insoluble precipitate; and

recovering the precipitate.

The term "soluble" in the preceding paragraph refers to species which are substantially more soluble in the reaction medium than is the precipitated product. In variants of the method, the ether is replaced by toluene or another aromatic solvent.

The diethanolamine precipitation technique described above is an example of another novel method, which is a method for recovering from ether solution a pinacol or pinanediol ester of a peptide boronic acid, comprising dissolving diethanolamine in the solution, allowing or causing a precipitate to form and recovering the precipitate. The invention encompasses variants of this methods in which another diol than pinacol or pinanediol is used.

The precipitated material, i.e. the "adduct", may be converted into the free organoboronic acid, for example by contacting it with an acid. The acid may be an aqueous acid, for example an aqueous inorganic acid, e.g. as described above. The precipitate may be dissolved, for example in an organic solvent, prior to being contacted with the acid.

The invention therefore provides a method for making an organoboronic acid, comprising converting its diolamine reaction product to the acid.

The acid resulting from the methods described in the previous two paragraphs may be converted to a salt of the acid with a multivalent metal, which salt may in turn be formulated into a pharmaceutical composition in oral dosage form.

3. Salt Synthesis

In general, the salts may be prepared by contacting the relevant peptide boronic acid with a strong base appropriate to form the desired salt. In the case of metal salts, the metal hydroxides are suitable bases (alternatively, metal carbonates might be used, for example), whilst salts with organic bases may be prepared by contacting the peptide boronic acid with the organic base itself. The preferred salts of the invention are acid salts (one -BOH proton replaced) and, to make acid salts with a monovalent cation, the acid and the base are suitably reacted in substantially equimolar quantities. Generally stated, therefore, the usual acid base molar ratio is substantially n:1, where n is the valency of the cation of the base.

In one procedure, a solution of the peptide boronic acid in a water-miscible organic solvent, for example acetonitrile or an alcohol (e.g. ethanol, methanol, a propanol, for example iso-propanol, or another alkanol), is combined with an aqueous solution of the base. The acid and the base are allowed to react and the salt is recovered. The reaction is typically carried out at ambient temperature (e.g. at a temperature of from 15 to 25°C), but an elevated temperature may be used, for example up to the boiling point of the reaction mixture but more usually lower, e.g. a temperature of up to 40°C or 50°C. The reaction mixture may be allowed to stand or be agitated (usually stirred).

The time during which the acid and the base are allowed to react is not critical but it has been found desirable to maintain the reaction mixture for at least one hour. A period of from one to two hours is usually suitable but longer reaction times are included in the invention.

The salt may be recovered from the reaction mixture by any suitable method, for example evaporation or precipitation. Precipitation may be carried out by adding an excess of a miscible solvent in which the salt has limited solubility. In one preferred technique, the salt is recovered by

evacuating the reaction mixture to dryness. The salt is preferably thereafter purified, for example by redissolving the salt before filtering the resulting solution and drying it, for example by evacuating it to dryness. The redissolution may be performed using water, e.g. distilled water. The salt may then be further purified, for example in order to remove residual water by further redissolution in a suitable solvent, which is advantageously ethyl acetate or THF followed by evaporating to dryness. The purification procedure may be carried out at ambient temperature (say, 15 to 25°C), or at a modestly elevated temperature, such as e.g. a temperature not exceeding 40°C or 50°C; for example the salt may be dissolved in water and/or solvent by agitating with or without warming to, for example, 37°C.

The invention includes a method for drying the salts of the invention and other peptide boronic acid salts, comprising dissolving them in a polar solvent, e.g. ethyl acetate or THF, and then evaporating to dryness, e.g. by evacuation.

Generally, preferred solvents for use in purifying the salts are ethyl acetate or THF, or perhaps another polar solvent.

A preferred general procedure for synthesising salts of Cbz-Phe-Pro-BoroMpg-OH is as follows:

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added the requisite base in solution in distilled water (190ml); the base is added as a 0.2M solution for a monovalent cation. The resultant clear solution is allowed to react for example by being left to stand or being agitated, for a usual period, in either case, of from one to two hours. The reaction is typically carried out at ambient temperature (e.g. 15-25°C) but alternatively the temperature may be elevated (e.g. up to 30°C, 40°C or 50°C). The reaction mixture is then evacuated to dryness under vacuum with its temperature not exceeding 37°C, typically to yield a white brittle solid or an oil/tacky liquid. The oil/tacky liquid is redissolved in the minimum amount of distilled water necessary (200ml to 4L), typically with warming (e.g. to 30-40°C), usually for up to 2 hours. The solution is filtered, suitably through filter paper, and evacuated to dryness, again with the temperature of the solution not exceeding 37°C, or freeze dried. The resultant product is dried under vacuum overnight to normally yield a white brittle solid. If the product is present as an oil or tacky solid then it is dissolved in ethyl acetate and evacuated to dryness to produce the product as a white solid. The white solid is typically a coarse, amorphous powder.

In variations of the aforegoing general procedure, the acetonitrile is replaced by another water-miscible organic solvent, notably an alcohol, as discussed above, especially ethanol, methanol, iso-propanol or another propanol.

Where a boronic acid salt is less soluble in a selected reaction medium for salt formation such that its direct preparation from the corresponding acid and base is inconvenient, the less soluble salt may be prepared from a salt more soluble in the reaction medium.

The invention provides also the use of a boronic acid to make a salt of the invention. Included also is a method of preparing a product of the invention, comprising contacting a boronic acid, e.g. of formula (I), (II) or (III), with a base capable of making such a salt.

The peptide boronic acid of formula (I) used to prepare the pharmaceutical preparations is typically of GLP or GMP quality, or in compliance with GLP (good laboratory practice) or GMP (good manufacturing practice); such acids are included in the invention.

Similarly the acids are usually sterile and/or acceptable for pharmaceutical use, and one aspect of the invention reside in a composition of matter which is sterile or acceptable for pharmaceutical use, or both, and comprises a peptide boronic acid of formula (I). Such a composition of matter may be in particulate form or in the form of a liquid solution or dispersion.

The intermediate acid may be in isolated form and such isolated acids are included in the invention, especially isolated acids which are a peptide boronic acid of formula (VIII):

X-(R)-Phe-(S)-Pro-(R)-Mpg-$B(OH)_2$ (VIII)

wherein X is H (to form NH_2) or an amino-protecting group.

One typical way of providing the intermediate acids is as a particulate composition consisting predominantly of such a peptide boronic acid, and these compositions are included in the invention. The peptide boronic acid often forms at least 75% by weight of the composition and typically at least 85% by weight of the composition, e.g. at least 95% by weight of the composition.

Another typical way of providing the intermediate acids is as a liquid composition consisting of, or consisting essentially of, a peptide boronic acid of formula (II) and a liquid vehicle in which it is dissolved or suspended. The liquid vehicle may be an aqueous medium, e.g. water, or an alcohol, for example methanol, ethanol, isopropanol, or another propanol, another alkanol or a mixture of the aforegoing.

The compositions of the intermediate acids are generally sterile. The compositions may contain the peptide boronic acid in finely divided form, to facilitate further processing.

Separation of Stereoisomers

The stereoisomers of a peptide boronic acid or a synthetic intermediate aminoboronate may be resolved in, for example, any known way. Accordingly, they may be resolved by chromatography (HPLC) or salt crystallisation.

Examples

The following compounds are referred to in the Examples:

TRI 50b = Cbz-Phe-Pro-BoroMpg-OPinacol.
TRI 50c = Cbz-Phe-Pro-BoroMpg-OH. This is the free acid of TRI 50b.

It is considered that the TRI 50b and TRI 50c featured in the examples are at least predominantly of the most active isomer, considered to be of RSR (DLL) configuration, as discussed above.

The solubility data presented in the examples were obtained from salt made using a modification of the salt preparation process described in the examples. The modified process differs from that described in the examples in that 100mg of TRI 50c was used as starting material, the product of the redissolution in water was dried by freeze drying and the filtration was carried out through a 0.2µm filter. The salt for which solubility data are presented is believed to contain about 85% of the most active isomer, considered to be of RSR configuration. When repeated with very pure active isomer salt obtained using the procedure described in the example from isomerically pure TRI 50c, the solubility data were the same as those presented within experimental error or very slightly higher.

EXAMPLE 1 – SYNTHESIS OF TRI 50C

APPARATUS

Throughout the following procedures, standard laboratory glassware and, where appropriate, specialised apparatus for handling and transferring of air sensitive reagents are used.

All glassware is heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen.

A. 3-METHOXYPROPENE

1 PROCEDURE

1.1 PREPARATION

To a mechanically stirred cooled solution under nitrogen with a gas outlet and fitted with a water condenser of allyl alcohol (107.8ml, 1.59mol) and dimethylsulphate (200ml, 1.59mol, 1.eq.) in 1,4-dioxane (1L) is added, portionwise NaH (60% dispersion in mineral oil, 63.5g, 1.59mol, 1eq.). Care is taken that the reaction temperature remains at or below room temperature and the reaction is stirred until effervescence has ceased.

1.2 PURIFICATION AND WORK-UP

The slurry is stirred, carefully, into ice (1L), and extracted with toluene (3x500ml). The organic phase is heated (mantle) with a fractionation column, to distil off at atmospheric pressure the methoxypropene, b.p. 45-60^{o}C. Heating should be observed to keep the vapour temperature in the 45-60^{o}C range, since unreacted allyl alcohol distils at 96-98^{o}C.

The resultant 3-methoxypropene must be stored at below 4^{o}C.

B. 3-METHOXYPROPYL BORONATE CATECHOL ESTER

1 PROCEDURE

1.1 PREPARATION

To 3-methoxypropene (120g, 1.66mol) in a 1l flask cooled in an ice bath and fitted with a condenser, is added, dropwise by dry transfer via a dropping funnel, catecholborane (199.6g, 1eq.) (which is prewarmed, if necessary, to give a liquid) and left overnight at room temperature. Careful addition of the catecholborane is necessary as the reaction can become violently exothermic. The mixture is heated at 60-70^{o}C for 24hrs. The mixture is allowed to cool to room temperature.

C. 3-METHOXYPROPYL BORONATE PINACOL ESTER

1 PROCEDURE

1.1 PREPARATION

To catechol 3-methoxypropaneboronate (1.66mol, from section B2) is added, at 0^{o}C, pinacol (126g, 1eq). The solution is stirred at 0^{o}C for 1hr. Remove the ice bath and leave at room temperature overnight.

1.2 PURIFICATION AND WORK-UP

To a 3l flask containing 1.5l hexane (lab. grade, not dried) transfer the solution from 3.1. Allow the catechol to precipitate out (storage at <4^{o}C for 1-2 hrs. facilitates this) and decant off the hexane

into a 3l separating funnel. Wash the precipitate with a further 500ml of hexane and add to the first hexane solution. Wash the hexane with water (2x500ml, analytical grade). Back extract each aqueous wash with (2x500ml) hexane. Dry the hexane layer with anhydrous $MgSO_4$. Filter (glass sinter, grade four).

Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

D. 4-METHOXY-1-CHLOROBUTYL BORONATE PINACOL ESTER

1 PURIFICATION OF REAGENTS

1.2.1 Dichloromethane

Add phosphorus pentoxide to dichloromethane at the rate of ca. 10 g per $100cm^3$ and leave to stand in a stoppered flask for at least 30 minutes. Distil the dichloromethane from the phosphorus pentoxide under a stream of dry nitrogen. The purified solvent is used immediately

1.2.2 Tetrahydrofuran

Distillation apparatus is set up containing tetrahydrofuran over sodium containing benzophenone (ca. 0.5 g per litre) as an indicator. If the colour of the solvent in the distillation flask is not blue add sodium (in oil) in small pieces, ca. 5 mm cubes until a blue colour develops. Distil the solvent from the sodium under a stream of dry nitrogen.

The purified tetrahydrofuran is used immediately and stored.

2 PROCEDURE

2.1 PREPARATION

To a solution (0.4M, in a 10l flask) of pinacol 3-methoxypropylboronate ester (150g, 0.750mol) in anhydrous cyclohexane (1250ml) and THF (625ml) (section 1.2.2) cooled to -20°C in a carbon tetrachloride/dry ice bath, is added dry DCM (section 1.2.1, 1.22eq., 58.5ml, 0.915mol). Added to this solution (with stirring, under stream of dry argon) dropwise, to maintain the temperature between -20 °C and -15 °C, is lithium diisopropylamide (1.11eq., 416ml, 0.833mol, diluted in 500ml THF) and then zinc chloride (0.5M solution in THF,1500ml) pre cooled in ice. The reaction is allowed to warm to room temperature overnight.

2.2 PURIFICATION AND WORK-UP

The reaction mixture is diluted in hexane (2l) and poured into cold 1M sulphuric acid (1l), stir for 15 mins, and then extract with hexane (2x500ml). Wash the combined extracts with saturated $NaHCO_3$ solution (1l), saturated NaCl solution (1l). Dry the combined hexane extracts with anhydrous $MgSO_4$.

Filter immediately with a grade four glass sinter.

Remove the solvent using a rotary evaporator at room temperature and with a vacuum of ca. 1 mm/Hg. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

E. 4-METHOXY-1-BIS (TRIMETHYLISILYL) AMINOBUTYL BORONATE PINACOL ESTER

1 PURIFICATION OF REAGENTS

1.1 Tetrahydrofuran

See section D, paragraph 1.2.2.

2 PROCEDURE

2.1 PREPARATION

A 0.33M solution of pinacol 4-methoxy-1-chlorobutaneboronate (150g, 0.60mol) in THF (1810ml) is added to a 0.5M solution of lithium hexamethyldisilazane (1N in hexane, 604ml, 1eq) in THF (603ml) at -78°C (dry ice/acetone bath) giving a final concentration of boronate at 0.2M. The reaction mixture is allowed to warm slowly to room temperature and is stirred for at least 12hrs.

2.2 PURIFICATION AND WORK-UP

Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

Hexane (laboratory grade, 1000ml) is added to yield a precipitate which is removed by washing with water (2x750ml, analytical grade). Back extract each aqueous phase with (500ml) hexane. Dry the hexane layer with anhydrous $MgSO_4$ and filter through a grade 4 glass sinter. The organic phase is concentrated using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

The residual oil is distilled under reduced pressure to give b.p. 80-104°C, 0.1 – 0.2 mmHg pinacol 4-methoxy-1-bis(trimethylsilyl)aminobutyl boronate.

F. 4-METHOXY-1-AMINOBUTYL BORONATE PINACOL ESTER

1. PURIFICATION OF REAGENTS

1.2.1 n-Hexane

Add calcium hydride to n-hexane at the rate of ca. 10 g per $100cm^3$ and leave to stand in a stoppered flask for at least 30 minutes. Distil the hexane from the calcium hydride under a stream of dry nitrogen. The purified solvent should be used immediately wherever possible but may be stored for up to 24 hours in a tightly stoppered flask.

1.2.2 Chloroform.

Add phosphorus pentoxide to chloroform at the rate of ca. 10 g per $100cm^3$ and leave to stand in a stoppered flask for at least 30 minutes. Distil the chloroform from the phosphorus pentoxide under a stream of dry nitrogen. The purified solvent should be used immediately wherever possible but may be stored for up to 24 hours in a tightly stoppered flask.

2 PROCEDURE

2.1 PREPARATION

To a 0.4M solution of pinacol 4-methoxy-1-bis(trimethylsilyl)aminobutane boronate (160g, 0.428mol) in dry hexane (1072ml, section 1.2.1) at -78°C (dry ice/acetone), is added HCl(4N, solution in dioxane, 322ml, 3eq.) from a measuring cylinder. The reaction is allowed to warm to room temperature overnight.

2.2 PURIFICATION AND WORK-UP

Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

Dry chloroform (2l, section 1.2.2) is added. The solution is then filtered through celite under nitrogen pressure in a closed system(grade four glass sinter). Organic phase is concentrated using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

G. Cbz-D-Phe-Pr -BoroMpg-OPinac (TRI 50b)

1 PURIFICATION OF REAGENTS

1.2.1 Tetrahydrofuran

See section D, paragraph 1.2.2.

2 PROCEDURE

2.1 PREPARATION

To a 0.5M solution of Cbz-D-Phe-Pro (0.515mol,204.5g,1eq) in THF (1042ml) is added N-methylmorpholine (56.8ml, 1eq.) and the solution cooled to -20°C (CCl_4/dry ice bath). $^iBuOCOCL$ (67ml,1eq, in 149ml THF, 3.5M) is added making sure the temperature stays in the range of -20 °C to -15°C. After 15 mins, to the mixture, is added by dry transfer a 1.36M solution of pinacol 4-methoxy-1-aminobutylboronate hydrochloride (150g, 0.57mol, 1.05eq) as a precooled solution in $CHCl_3$ (416ml), then Et_3N (75.3ml,1.05eq) is added. The reaction is allowed to warm to room temperature and stirred for at least 2hrs.

2.2 PURIFICATION AND WORK-UP

Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

The residue is dissolved in ethyl acetate (1500ml) and washed with HCl (0.2M, 2x500ml),back extract the combined HCl washes with ethyl acetate (500ml) and combine with ethyl acetate layer. Wash combined ethyl acetate with water (1000ml), back extract the water wash with 500ml of ethyl acetate combined with ethyl acetate layer, $NaHCO_3$ (saturated aqueous, 2x1000ml) and NaCl (saturated aqueous, 500ml). To the organic phase is added dried magnesium sulphate until it flocculates, the flask stoppered tightly and left to stand for at least 30 minutes. Remove the magnesium sulphate by filtration through a glass sinter, (grade four). Remove the solvent using a rotary evaporator at room temperature and with a vacuum of ca. 1 mm/Hg. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

Leave overnight on high vacuum.

The desired crude product as a foamy solid.

2.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

2.3.1 NMR Analysis

The TRI 50b should be checked by ^{1}H NMR spectroscopy. Signals should be observed as follows:-

δ_{400}	Signal Pattern	Assignment
7.82	1H, broad	NH
7.40-7.20	10H, multiplet	2xPh
5.7	1H, broad	NH
5.17-5.08	2H, dd, J=7.54Hz	Ph$\underline{CH_2}$O
4.48-4.44	2H, multiplet	Pro α-CH, Pheα-CH
3.46	1H, multiplet	Pro-C4
3.27	2H, multiplet	$\underline{CH_2}$OMe
3.22	3H, singlet	Ome
2.99	2H, multiplet	Ph$\underline{CH_2}$
2.63	1H, multiplet	CHB
2.59-2.23	4H, multiplet	Pro-C3, Pro-C2
1.60	4H, multiplet	$\underline{CH_2}\underline{CH_2}$
1.20	12H, singlet	pinacol

The TRI 50b should be checked by ^{13}C NMR spectroscopy. ^{o}C Signals should be observed as follows:-

δ_{400}	Signal Pattern	Assignment
171	quaternary	O-$\underline{C}$O-N
156	quaternary	CH-$\underline{C}$O-N
136	quaternary	Ph
130-126	CH	aromatics
81.5	quaternary	$\underline{C}Me_2$
73	CH_2	$\underline{C}H_2$OMe
67.26	CH_2	PhCH_2O
58.3	CH	Pro-αCH
57.94	CH_3	Ome
54.46	CH	Phe-αCH

46.77	CH_2	Pro-4-CH_2
38.76	CH_2	Ph$\underline{C}H_2$CH
27.84-27.4	2x CH_2	$\underline{C}H_2\underline{C}H_2CH_2OMe$
25.23-24.9	4xCH_3	pinacol, major isomer
24.07	CH_2	Pro-3- CH_2

Due to the presence of impurities other signals will be observed also.

2.3.2 HPLC Analysis

[note: a) tripeptide cannot be recovered from aqueous solution. b) Dipeptide elutes at solvent front and does not give a peak in this system]

- Column: Reverse phase C-18 ODS (octadecylsilane) 2.5μm, 150x4.6mm
- Flow: 1.5ml/min.
- Detection: UV at 225 nm
- Injection volume: 0.02ml
- Solvent A: 20% MeCN in analytical grade water.
- Solvent B: 55% MeCN in analytical grade water.
- Gradient: Linear from 20 to 90% mobile phase B over initial 15 minutes. Conditions maintained at 90% mobile phase B for a further 10 minutes. Linear to 100% B over 10mins, conditions maintained at 100% B for 5 mins then re-equilibrated to initial conditions.

Component	Rt (min)
Z-D-Phe-Pro-(S)-boroMpgOPinacol	16(+-1)
Z-D-Phe-Pro-(R)-boroMpgOPinacol	17(+-1)

H. Cbz-D-Phe-Pro-BoroMpg-OH (TRI 50c)

To a solution of TRI 50b (rmm 608) in acetone (1g/10ml), is added phenyl boronic acid (1.01 equivalent, rmm 120) and the solution stirred by a mechanical stirrer. To the solution is slowly added ammonium hydroxide solution, (5%, pH adjusted to pH 9 by HCl, same volume as acetone). Some cloudiness may develop.

Hexane (equal volume to total acetone and ammonium hydroxide) is added and the solution stirred rapidly for four hours. Stirring is stopped and the hexane layer decanted (if an oil forms, this is kept with the aqueous layer by washing with a small volume of acetone). Hexane (same volume) is added, stirred for 10mins, decanted and repeated.

The aqueous layer is concentrated to about 1/3 volume by rotary evaporator with card-ice cold finger (water bath <35°C). Some oil may form on the side of the flask. The solution is then acidified (0.1N HCl) to pH 3 (care: do not acidify below pH 3), and extracted by EtOAc (2x same as original acetone volume). Sample can be concentrated without drying to give a foam, yield ~70%.

EXAMPLE 2 – ALTERNATIVE CONVERSION OF TRI 50B TO TRI 50C

1. Approximately 300 g of TRI 50b were dissolved in approximately 2.5 L diethylether.
2. Approximately 54 ml diethanolamine were been added, the mixture was refluxed at 40 °C.
3. The precipitated product was removed, washed several times with diethylether and dried.
4. The dry product was dissolved in $CHCl_3$. Hydrochloric acid (pH 1) was added and the mixture was stirred approximately 1h at room temperature.
5. The organic layer was removed and washed with NH_4Cl solution.
6. The organic solvent was distilled off and the residual solid product was dried.

Typical yield: Approximately 230 g

EXAMPLE 3 - SEPARATION OF DIASTEREOMERS

The R-Mpg and S-Mpg isomers of TRI 50b and TRI 50c are separated chromatographically as summarised below.

A solution of 5gm/ml of TRI 50b in acetonitrile is prepared and 10 μL is injected to a Lichrosphere™ cyano column and eluted with a gradient of n-hexane and tetrahydrofuran with monitoring at 206nM. Analysis of the UV chromatogram indicates TRI 50b isomer I ('R' configuration at α-aminoboronate centre) elutes at (retention time) Rt 11.1 minutes; TRI 50b isomer II ('S' configuration at α-aminoboronate centre) elutes at Rt 13.7minutes.

Following the same procedure, TRI 50c isomer I ('R' configuration at α-aminoboronate centre) elutes at (retention time) Rt 21.2 minutes; TRI 50b isomer II ('S' configuration at α-aminoboronate centre) elutes at Rt 22.2 minutes.

Conditions:

Column: Licrosphere Cyano Merck.4.6 x 250mm, 5μ.
Solvent A : n-Hexane
Solvent B THF
Gradient 0-100% B over 25 minutes

Monitor UV at 206nm

Sample concentration 5mg/ml.

The results are shown in the chromatogram of Fig 1.

The above microanalytical data show C and N amounts below calculated, suggesting the samples might have contained unremoved water.

EXAMPLE 4 - PREPARATION OF LITHIUM SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added LiOH as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 500ml distilled water necessary with light warming for about 20 minutes. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

Yield 17.89g.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)	Metal % Found (Calc.)
57.14 (61.03)	6.60 (6.64)	7.34 (7.90)	2.07 (2.03)	Li 1.26 (1.31)

EXAMPLE 5 – UV/VISIBLE SPECTRA OF LITHIUM SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. The salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \varepsilon cl$ where A is the absorbance

C is the concentration

I the path length of the UV cell

and ε is the extinction coefficient.

Extinction coefficient: 451

EXAMPLE 6 – AQUEOUS SOLUBILITY OF LITHIUM SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material. The lithium salt was comparatively soluble and so was redissolved at 50mg/ml in the same manner previously described.

Solubility when dissolved at 25mg/ml: 43mM (23 mg/ml).

Solubility when dissolved at 50mg/ml: 81mM (43 mg/ml).

EXAMPLE 7 - PREPARATION OF SODIUM SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added NaOH as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 500ml distilled water with light warming for about 15-20 minutes. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid. The product may be present as an oil or tacky solid due to residual water, in which case it is dissolved in ethyl acetate and evacuated to dryness to produce the product as a white solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

Yield: Over 50%.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)	Metal % Found (Calc.)
59.93 (59.24)	6.47 (6.44)	7.31 (7.67)	1.91 (1.98)	Na 3.81 (4.20)

EXAMPLE 8 – UV/VISIBLE SPECTRA OF SODIUM SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. The salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \varepsilon cl$ where A is the absorbance
C is the concentration
l the path length of the UV cell
and ε is the extinction coefficient.

Extinction coefficient: 415.

EXAMPLE 9 – AQUEOUS SOLUBILITY OF SODIUM SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material. The sodium salt was comparatively soluble and so was redissolved at 50mg/ml in the same manner previously described.

Solubility when dissolved at 25mg/ml: 44mM (25 mg/ml).
Solubility when dissolved at 50mg/ml: 90mM (50 mg/ml).

EXAMPLE 10 - PREPARATION OF POTASSIUM SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added KOH as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 1L distilled water with warming to 37°C for about 2 hours. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid.
Yield: 14.45 mg.

The salt was then dried under vacuum over silica to constant weight (72 h).

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)	Metal % Found (Calc.)
54.84 (57.55)	6.25 (6.26)	7.02 (7.45)	2.01 (1.92)	K 4.29 (6.94)

EXAMPLE 11 – UV/VISIBLE SPECTRA OF POTASSIUM SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \varepsilon cl$ where A is the absorbance
C is the concentration
l the path length of the UV cell
and ε is the extinction coefficient.

Extinction coefficient: 438.

EXAMPLE 12 – AQUEOUS SOLUBILITY OF POTASSIUM SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material.

Solubility when dissolved at 25mg/ml: 29mM (16 mg/ml).

EXAMPLE 13 – PREPARATION OF ZINC SALT OF TRI 50C

The relative solubility of zinc hydroxide is such that, if the hydroxide had been used to prepare the corresponding TRI 50c salt using the procedure of Example 4, they would not have resulted in homogeneous salt formation. A new method was therefore developed to prepare the zinc salt, as described in this and the next examples.

TRI 50c sodium salt (2.24g, 4.10mM) was dissolved in distilled water (100ml) at room temperature and zinc chloride in THF (4.27ml, 0.5M) was carefully added with stirring. A white precipitate that immediately formed was filtered off and washed with distilled water. This solid was dissolved in ethyl acetate and washed with distilled water (2 x 50ml). The organic solution was evacuated to dryness

and the white solid produced dried over silica in a desiccator for 3 days before microanalysis. Yield 1.20g.

^{1}H NMR 400MHz, δ_H(CD_3OD) 7.23-7.33 (20H, m, ArH), 5.14 (4H, m, $PhCH_2O$), 4.52 (4H, m, αCH), 3.65 (2H, m), 3.31 (12H, m), 3.23 (6H, s, OCH_3), 2.96 (4H, d, *J*7.8Hz), 2.78 (2H, m), 2.58 (2H, m), 1.86 (6H, m), 1.40 (10H, m).

^{13}C NMR 75MHz 393K δ_C(CD_3OD) 178.50, 159.00, 138.05, 137.66, 130.54, 129.62, 129.50, 129.07, 128.79, 128.22, 73.90, 67.90, 58.64, 58.18, 56.02, 38.81, 30.06, 28.57, 28.36, 25.29.

FTIR (KBr disc) ν_{max} (cm^{-1}) 3291.1, 3062.7, 3031.1, 2932.9, 2875.7, 2346.0, 1956.2, 1711.8, 1647.6, 1536.0, 1498.2, 1452.1, 1392.4, 1343.1, 1253.8, 1116.8, 1084.3, 1027.7, 916.0, 887.6, 748.6, 699.4, 595.5, 506.5.

EXAMPLE 14 - PREPARATION OF ARGININE SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added arginine as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 2L distilled water with warming to 37°C for 2 hours. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

Yield: 10.54g.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)
52.47 (56.65)	7.12 (7.20)	15.25 (14.01)	1.52 (1.54)

EXAMPLE 15 – UV/VISIBLE SPECTRA OF ARGININE SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes

of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \varepsilon cl$ where A is the absorbance

C is the concentration

l the path length of the UV cell

and ε is the extinction coefficient.

Extinction coefficient: 406.

EXAMPLE 16 – AQUEOUS SOLUBILITY OF ARGININE SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material.

Solubility when dissolved at 25mg/ml: 14mM (10 mg/ml).

EXAMPLE 17 - PREPARATION OF LYSINE SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added L-lysine as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 3L distilled water with warming to 37°C for 2 hours. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid. The product may be present as an oil or tacky solid (due to residual water), in which case it is then dissolved in ethyl acetate and evacuated to dryness to produce the product as a white solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

Yield: 17.89.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)
57.03 (59.11)	7.43 (7.36)	10.50 (10.44)	1.72 (1.61)

EXAMPLE 18 – UV/VISIBLE SPECTRA OF LYSINE SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \varepsilon cl$ where A is the absorbance
C is the concentration
l the path length of the UV cell
and ε is the extinction coefficient.

Extinction coefficient: 437.

EXAMPLE 19 – AQUEOUS SOLUBILITY OF LYSINE SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material.

Solubility when dissolved at 25mg/ml: 13mM (8.6 mg/ml).

EXAMPLE 20 - PREPARATION OF N-METHYL-D-GLUCAMINE SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added N-methyl-D-glucamine as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 500ml distilled water with light warming for about 20 minutes. The solution is filtered through filer paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C, or freeze dried. The resultant product is dried under vacuum overnight to normally yield a white brittle solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

Yield: 21.31g.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)
56.67 (56.67)	7.28 (7.41)	7.74 (7.77)	1.63 (1.50)

EXAMPLE 21 – UV/VISIBLE SPECTRA OF N-METHYL-D-GLUCAMINE SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \varepsilon cl$ where A is the absorbance
C is the concentration
l the path length of the UV cell
and ε is the extinction coefficient.

Extinction coefficient: 433.

EXAMPLE 22 – AQUEOUS SOLUBILITY OF N-METHYL-D-GLUCAMINE SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt was observed to fully dissolve. The salt was comparatively soluble and so was redissolved at 50mg/ml in the same manner previously described.

Solubility when dissolved at 25mg/ml: 35mM (25 mg/ml).
Solubility when dissolved at 50mg/ml: 70mM (50 mg/ml).

EXAMPLE 23 – ALTERNATIVE PREPARATION OF ARGININE SALT OF TRI50C

The arginine salt is formed simply by adding a slight molar excess of L-arginine to a solution of 0.2-0.3mmol of TRI50c in 10ml of ethyl acetate. The solvent is evaporated after one hour, and the residue is triturated twice with hexane to remove excess arginine.

EXAMPLE 24 – SOLUBILITY OF TRI50C

The UV/visible spectra of TRI50c and its solubility were obtained as described above in relation to the salts. The solubility of TRI50c when dissolved at 50mg/ml was 8mM (4mg/ml).

^{13}C NMR 75MHz 393K $\delta_C(CD_3C(O)CD_3)$ 206.56, 138.30, 130.76, 129.64, 129.31, 129.19, 129.09, 128.20, 128.04, 74.23, 73.55, 67.78, 58.76, 56.37, 56.03, 48.38, 47.87, 39.00, 25.42, 25.29.
FTIR (KBr disc) ν_{max} (cm^{-1}) 3331.3, 3031.4, 2935.3, 2876.9, 2341.9, 1956.1, 1711.6, 1639.9, 1534.3, 1498.1, 1453.0, 1255.3, 1115.3, 1084.6, 1027.6, 917.3, 748.9, 699.6, 594.9, 504.5, 467.8.

EXAMPLE 25 – ANALYSIS OF SODIUM AND ZINC SALTS OF TRI 50C

The following salts were prepared using a boronate:metal stoichiometry of n:1, where n is the valency of the metal.

A. Sodium Salt

Analytical data

HPLC or LC/MS: HPLC betabasic C18 Column, CH_3CN, Water

Estimated Purity: >95% by UV (λ_{215nm})

Micro analysis:

	Calcd.	*Found.*
C:	59.24	59.93
H:	6.44	6.47
N:	7.67	7.31
Other: B:	1.98	1.91
Na:	4.20	3.81

Physical Properties

Form: Amorphous solid

Colour: White

Melting Point: N/A

Solubility: Soluble in aqueous media *ca*~50mg/ml

M_w: 547.40

B. Zinc Salt

Analytical data

HPLC or LC/MS: HPLC betabasic C18 Column, CH_3CN, Water

Estimated Purity: >95% by UV (λ_{215nm})

Micro analysis:

	Calcd.	*Found.*
C:	58.21	56.20
H:	6.33	6.33
N:	7.54	7.18
Other: B:	1.94	1.84
Zn:	5.87	7.26

Physical Properties

Form: Amorphous solid

Colour: White

Melting Point: N/A

Solubility: Soluble in aqueous media *ca*~2mg/ml

M_w: 1114.18

Notes: The trigonal formula of the acid boronate is used in the calculated microanalyses. It is believed that a lower sodium salt solubility is reported in example 8 because the salt tested in example 8 had lower chiral purity.

Conclusion

The sodium and zinc salts have all been prepared with a stoichiometry of, respectively, one metal ion to one molecule of TRI 50c and one metal ion to two molecules of TRI 50c. The value found for the sodium salt is close to that calculated for this 1:1 stoichiometry. For the zinc salt an excess of zinc was found; nonetheless, the zinc salt comprises a significant proportion of acid boronate.

EXAMPLE 26 - STABILITY

An assay of TRI 50c and its sodium and lysine salts before and after drying.

Method

TRI 50c and its Na, Ca and Lys salts were weighed into HPLC vials and stored in a desiccator over phosphorus pentoxide for 1 week. For sample analysis, 5 mg of dried and non-dried material was weighed in a 5 mL volumetric flask and dissolved in 1 mL acetonitrile and filled up with water to 5 mL.

The compounds were investigated by HPLC. For the impurity profiles, an HPLC peak area percentage was calculated. The results are shown in Table 1.

Table 1

Compound	Amount [µg/mL]	Purity (% area)
TRI 50c dry	1000.0	82.00
TRI 50c non-dried	947.3	85.54
TRI 50c Na salt dry	1024	98.81
TRI 50c Na salt non-dried	1005.8	98.61
TRI 50c Lys salt dry	813.3	90.17
TRI 50c Lys salt non-dried	809.8	92.25

The purity of the acid was lowered by the drying process but the purity of the salts was less affected; the purity of the sodium salt was not significantly reduced. Large differences in response factors will reduce the actual impurity levels, however.

This example indicates that the salts of the invention, particularly the metal salts, e.g. alkali metal salts, are more stable than the acids, notably TRI 50c.

EXAMPLE 27 - STABILITY

This Example compares the stability of TRI 50c and TRI 50c lysine salt when filled into enteric-coated hard gelatin capsules.

Compound	Packing	Climatic conditions 1.5 month [0)]	Purity (HPLC %Area) T0	Purity (HPLC %Area)[3] T1
TRI50c	capsules in blister	25°C / 60% r.h. [4]	99	73.9
TRI50c	capsules in blister	40°C / 75% r.h	99	73.9
TRI50c	capsules[1]	40°C / 75% r.h	99	75.3
TRI50c Lysine Salt	capsules in blister	25°C / 60% r.h.	90.2[2)]	90.5
TRI50c Lysine Salt	capsules in blister	40°C / 75% r.h	90.2[2]	91.8
TRI50c Lysine Salt	capsules[1]	40°C / 75% r.h	90.2[2]	90.6

Notes:

0) 1.5 month storage at given conditions, samples were then stored at room temperature until analytical testing.

1) capsules stored at the respective climatic conditions without blister.

2) purity of the batch before storage.

3) purity of the stored batch (capsules were poured out, the contents of the capsules were then analyzed).

4) r.h. = relative humidity

Conclusion

There was no significant difference in the purities of the lysine salt at T_0 and T_1.

EXAMPLE 28 - PARTICLE FORM

TRI 50c and certain of its salts were investigated by microscopy and X-ray diffraction. The salts are sodium, potassium, lithium, lysine, arginine and glucamine.

A. Material and methods

A.1 Microscopic Digital Photographs

Microscopic equipment: Leica® Type 090-135.002

Digital Camera: Nikon® Coolpix 990

A.2 X-Ray diffraction

Equipment: Bruker®AX, Typ "DIFFRAC 5000"

B Results

B.1 Microscopic Digital Photographs

Various shapes for the solid powder were detected. No hint of crystallinity was observed.

B.2 X-Ray diffraction

It is evident from the X-ray diffraction patterns that predominantly amorphous modifications are present for the investigated compounds.

C. Conclusion

The microscopic images show that the particles are very coarse. No crystal appearance could be detected which was confirmed by X-ray powder diffraction where no evidence of crystal structures could be detected.

EXAMPLE 29 - TRI 50B INHIBITION OF PLATELET PROCOAGULANT ACTIVITY

Platelet pro-coagulant activity may be observed as the increase, in rate of activation of prothrombin by factor Xa in the presence of factor Va upon the addition of platelets pretreated with thrombin, caused by thrombin alone, collagen alone or a mixture of thrombin and collagen. This property is due to an increase in anionic phospholipid on the surface of the platelet with concomitant release of microvesicle from the surface. This is an essential physiological reaction and people whose platelets have reduced ability to generate procoagulant activity (Scott syndrome) show an increased tendency for bleeding.

Method:

Washed platelets were treated with either 1.15nM thrombin, 23μg/ml collagen or a mixture of both at the same concentration at 37°C. TRI 50b was added either for 1 minute prior to the addition of activator or immediately after the incubation with activator. Platelet procoagulant activity was determined as described previously (Goodwin C A et al, *Biochem J.* **1995** 8, 308: 15-21).

TRI 50b proved to be a potent inhibitor of platelet procoagulant activity with IC_{50}'s as summarised below:

Table 2: Influence of TRI 50b on the induction of platelet procoagulant activity by various agonists:

Table 2

Agonist	Fold acceleration without TRI 50b	IC50 plus pre-incubation (nM)	IC50 without incubation (nM)
Thrombin	30	8	3000
Collagen	45	200	300
Thrombin/Collagen	110	3	80

Table 2 records, for example, that when platelets were treated with thrombin they caused a 30-fold acceleration of the rate of activation of prothrombin in comparison with control platelets. Treatment with TRI 50 reduced such acceleration by half at the various TRI 50 concentration levels given. The significant potency of TRI 50 is evidenced by the fact that the IC_{50} values are in the nanomolar range.

TRI 50b does not have an effect on ADP, collagen or epinephrine induced aggregation of washed platelets.

EXAMPLE 30 - RABBIT EXTRACORPOREAL SHUNT MODEL

Introduction

The technique describes an animal model in which a platelet rich thrombus is produced. The activity of TRI 50b and heparin are compared.

The carotid artery and jugular vein of anaesthetised rabbits were used to create an extracorporeal circuit containing a suspended foreign surface (silk thread). Thrombus deposition is initiated by creation of high sheer stress turbulent arterial blood flow, platelet activation, followed by coagulation in the presence of thrombogenic surfaces. Histopathological studies have shown that the thrombus is platelet rich.

Materials and Methods

Animals:

NZW rabbits (males 2.5-3.5 kg) were used. The animals were allowed food and water up to the induction of anaesthesia.

Anaesthesia:

Animals were premedicated with fontanel/fluanisone (Hypnorm) 0.15 ml total by intramuscular injection. General anaesthesia was induced with methohexitone (10 mg/ml) to effect, followed by endotracheal intubation. Anaesthesia was maintained with isoflurane (1-2.0 %) carried in oxygen /nitrous oxide.

Surgical Preparation:

The animals were placed in dorsal recumbency and the ventral cervical region prepared for surgery. The left carotid artery and right jugular vein were exposed. The artery was cannulated with a large Portex® catheter (yellow gauge), cut to a suitable length. The vein was cannulated with a Silastic® catheter. The shunt comprised of a 5 cm length of 'auto analyser' line (purple /white gauge). Joins to the shunt on the arterial side were made with intermediate size Silastic® tubing. The shunt was filled with saline before exposure to the circulation. The right femoral artery was cannulated for the measurement of blood pressure.

Thread Preparation and insertion:

The central section of the shunt contained a thread 3 centimetres in length. This consisted of 000 gauge Gutterman sewing silk so as to give four strands with a single knot at the end. (The knot section was outside the shunt).

Blood Flow

Blood flow velocity was determined by use of 'Doppler' probes (Crystal Biotech). A silastic probe was positioned over the carotid artery at the point of insertion of the arterial catheter. Flow was recorded on a chart recorder using heat sensitive paper.

RESULTS

Table 3

TREATMENT	DOSE	THROMBUS WEIGHT AFTER 20 minute run	ANTITHROMBOTIC ACTIVITY
Control	N/A	22.4 ±2.2 mg (n=5)	
TRI 50b	10mg/kg iv	9.78 ±1.9 mg(n=5)	Active
	3.0mg/kg iv	15.3 ±2.2 mg(n=5)	Active
HEPARIN	100 u/kg iv	22.9 ±1.65 mg(n=4)	Inactive
	300 u/kg iv	10.5 ±1.4 mg (n=4)	Active (Severe bleeding)

Discussion

Table 3 shows that, under high arterial shear conditions, a TRI 50b dose of 3mg/kg to 10mg/kg iv significantly inhibits thrombus formation without bleeding, whereas a heparin dose within the normal clinical range for treating venous thrombosis (100u/kg iv heparin) was ineffective. The higher dose of heparin, though active, caused severe bleeding. These results, which show TRI 50b effectively inhibiting arterial thrombosis without causing bleeding, are consistent with TRI 50b inhibiting platelet procoagulant activity. In contrast, the thrombin inhibitor heparin, when administered at an

approximately equi-effective dose (in terms of inhibition of arterial thrombosis), produced the severe bleeding normal when thrombin inhibitors are used to treat arterial thrombosis.

EXAMPLE 31 – COMPARISON OF BLEEDING TIMES

The aim of the study was to compare the bleeding times of heparin with TRI 50b in a suitable model. It is accepted that heparin is a poor inhibitor of platelet procoagulant activity (*J. Biol. Chem.* **1978** Oct 10; 253(19):6908-16; Miletich JP, Jackson CM, Majerus PW1: *J. Clin. Invest.* **1983** May; 71(5):1383-91).

Bleeding times were determined in a rat tail bleeding model following intravenous administration of heparin and TRI 50b. The doses employed were chosen on the basis of their efficacy in the rat Wessler and dynamic models and were as follows:

TRI 50b: 5 and 10 mg/kg
Heparin: 100 units/kg

MATERIALS AND METHODS

Anaesthesia

Rats were anaesthetised with sodium pentabarbitone at 60 mg/kg (2.0 ml/kg of 30 mg/ml solution by ip. injection). Supplemental anaesthetic was given ip. as required.

Surgical preparation

A jugular vein was cannulated for the administration of test compound. The trachea was also cannulated with a suitable cannula and the animals allowed to breathe 'room air' spontaneously.

Compound administration

These were given in the appropriate vehicle at 1.0 ml/kg intravenously. Heparin was administered in saline, whilst TRI 50b was dissolved in ethanol, and then the resultant solution added to water for injection (1 part ethanol to 5 parts water).

Technique

Two minutes following compound administration the distal 2mm of the animal's tail was sectioned with a new scalpel blade and the tail immersed in warm saline (37°C) contained in a standard 'universal' container, so that the blood stream was clearly visible. The bleeding time recording was started immediately following transection until the cessation of blood flow from the tip of the tail. A period of 30 seconds was allowed after the blood flow from the tail had stopped to ensure that

bleeding did not re-commence, if bleeding did start again the recording time was continued for up to a maximum of 45 minutes.

Results

Table 4 gives a summary of the bleeding results and shows the increases above base line values.

Table 4

Summary table of bleeding results

Treatment	Bleeding time min (± SEM†)
Saline	5.1 ± 0.6
Heparin 100 u/kg iv	>40*
TRI 50b 5 mg/kg iv	11.3 ± 1.2
TRI 50b 10 mg/kg iv	30.4 ± 5.2

*Severe bleeding in all animals, with no cessation after 40 minutes.

†SEM = standard error of the mean

Discussion

The results show that TRI 50b was superior to heparin (produced less bleeding) at all doses. It should be noted that when 100 u/kg heparin is compared with 5 mg/kg TRI 50b, heparin-treated animals bled more extensively than those receiving TRI 50b; it was previously established (Example 25) that heparin at a dose of 100 u/kg is a less effective inhibitor of arterial thrombosis than TRI 50b at a dose of 3.0 mg/kg. Heparin is primarily a thrombin inhibitor and a poor inhibitor of platelet procoagulant activity; the results are therefore consistent with TRI 50b exerting anti-coagulant activity by inhibition of platelet coagulant activity in addition to thrombin inhibiting activity.

EXAMPLE 32 - TRI 50B AS A PRODRUG FOR TRI 50C: PHARMACOKINETICS AND ABSORPTION

MATERIALS AND METHODS

Animals

Rats, body weight circa 250-300g were used. The animals were fasted only on the day of use for the iv stage. Animals were fasted on the night prior to study for the oral and intraduodenal studies, water was allowed up to the time of anaesthesia.

Table 5:

iv phase

Treatment	Dose mg/kg iv	n
TRI 50b	1.0mg/kg	3
TRI 50c	1.0mg/kg	3

Table 6:

oral phase

Treatment	Dose mg/kg po	n
TRI 50b	20mg/kg	2
TRI 50c	20mg/kg	2

Table 7:

intraduodenal phase

Treatment	Dose mg/kg po	n
TRI 50b	20mg/kg	3
TRI 50c	20mg/kg	3

Dose

Formulation (TRI 50b/TRI 50c)

These were dosed in a formulation prepared as follows: 48 mg/ml of TRI 50b is dissolved in ethanol: PEG 300 (2:3 vol: vol). Just before administration, 5 volumes of this solution is mixed with 3 volumes of 5% kollidon 17 8F.

i.v. Phase

Both compounds were given at a dose of 1.0mg/kg iv.

Oral Phase

1) Both compounds were dosed by oral gavage at 20mg/kg.

2) As 1) but directly into the duodenum.

The compounds were dosed in a PEG/ethanol/kollidon formulation which was prepared immediately before, as described immediately under the heading "Dose": Stock 15.0mg/ml. This was dosed at 1.33ml/kg (equivalent to 30mg/kg).

Meth ds

Oral gavage

Rats were dosed at 20mg/kg. Approximately 30 minutes following dosing the rats were anaesthetised.

Intraduodenal administration

The compounds were instilled directly into the duodenum after anaesthesia and surgical procedures had been completed.

Blood sampling

i.v. Phase

A pre dose sample was taken followed by: 0, 2, 5, 10, 20, 30, 40, 60 and 90 minutes post dose.

Oral phase

Blood (0.81ml) was taken from the carotid cannula into (0.09ml) of 3.8% w/v tri sodium citrate following anaesthesia and surgery. The first samples were taken one-hour post dose. Then at, 1.5, 2, 4 hours post dose.

Intraduodenal phase

Blood samples were taken: Pre dose, then at 0.25, 0.5, 0.75, 1.0, 2, 3 and 4 hours post dosing.

Plasma

This was obtained by centrifugation (3000 RPM for 10 min) and stored at -20°C prior to analysis.

RESULTS

PHARMACOKINETIC ANALYSIS

Intravenous phase

Table 8:

i.v. pharmacokinetic data

	TRI 50b	TRI 50c
Elimination half life: minutes	35 minutes	36.6 minutes
Area under curve	1.68	1.48
Mean Residence Time	46 minutes	45 minutes
Clearance: ml/min/kg	10	11.3
Volume Distribution Litres/kg	0.5	0.59
Max Plasma Concentration (observed)	2.24	2.35

The following results are represented in Figures 2 to 2:

Fig 2: intravenous phase clearance and kinetics following a single dose of TRI 50b or its free acid (TRI 50c). The figure shows the observed assay data.

Fig 3: oral phase clearance and kinetics following dosing with TRI 50b or its free acid (TRI 50c).

Fig 4: oral phase clearance and kinetics following intraduodenal dosing with TRI 50b or its free acid (TRI 50c).

CONCLUSION

When given by the intraduodenal route TRI 50b achieved a higher bioavailability (peak plasma concentration) than the free acid. The i.v. kinetics were similar for both compounds. The data are consistent with TRI 50b being rapidly hydrolysed in plasma to TRI 50c and with TRI 50c being the active principle.

The results of examples 29 to 32 indicate that administration of TRI 50c as a salt will provide a way to treat arterial thrombosis and/or venous thrombosis.

EXAMPLE 33 – Human Clinical Studies

In human clinical volunteer studies with doses of up to 2.5mg/kg i.v. (dosages which significantly prolong the thrombin clotting time), TRI 50b had no effect on Simplate bleeding time (i.e. bleeding time measured using a Simplate® bleeding time device).

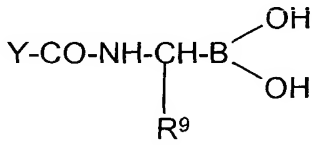
It will be appreciated from the foregoing that the invention provides boronic acid salts useful for pharmaceutical purposes and which feature one or more of the following attributes: (1) improved amount of oral bioavailability; (2) improved consistency of oral bioavailability; (3) improved stability; and (4), in any event, not suggested by the prior art.

The selection of active ingredient for a pharmaceutical composition is a complex task, which requires consideration not only of biological properties (including bioavailability) but also of physicochemical properties desirable for processing, formulation and storage. Bioavailability itself is dependent on various factors, often including in vivo stability, solvation properties and absorption properties, each in turn potentially dependent on multiple physical, chemical and/or biological behaviours.

Advantageously, at least preferred products of the invention have adequate absorption and bioavailability. For commercial utility, a product having less good solubility may be selected by virtue of a superior overall combination of properties.

CLAIMS

1. A salt of a boronic acid of formula (I):



(I)

wherein

Y comprises a hydrophobic moiety which, together with the aminoboronic acid residue $-NHCH(R^9)-B(OH)_2$, has affinity for the substrate binding site of thrombin; and

R^9 is a straight chain alkyl group interrupted by one or more ether linkages and in which the total number of oxygen and carbon atoms is 3, 4, 5 or 6 or R^9 is $-(CH_2)_m-W$ where m is from 2, 3, 4 or 5 and W is –OH or halogen (F, Cl, Br or I).

2. A salt of claim 1 wherein R^9 is an alkoxyalkyl group.

3. A salt of claim 1 or claim 2 wherein YCO- comprises an amino acid which binds to the S2 subsite of thrombin, the amino acid being N-terminally linked to a moiety which binds the S3 subsite of thrombin.

4. A salt of claim 1 or claim 2 wherein Y is an optionally N-terminally protected dipeptide which binds to the S3 and S2 binding sites of thrombin and the peptide linkages in the acid are optionally and independently N-substituted by a C_1-C_{13} hydrocarbyl optionally containing in-chain or in-ring nitrogen, oxygen or sulfur and optionally substituted by a substituent selected from halo, hydroxy and trifluoromethyl.

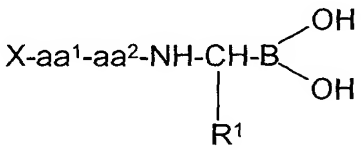
5. A salt of claim 4 wherein said dipeptide is N-terminally protected and all the peptide linkages in the acid are unsubstituted.

6. A salt of claim 4 or claim 5 wherein S3-binding amino acid residue is of R configuration, the S2-binding residue is of S configuration, and the fragment $-NHCH(R^9)-B(OH)$ is of R configuration.

7. A salt of any of claims 1 to 6 wherein the boronic acid has a Ki for thrombin of about 100 nM or less.

8. A salt of claim 7 wherein the boronic acid has a Ki for thrombin of about 20 nM or less.

9. A salt of a boronic acid of formula (II):



(II)

where:

X is H (to form NH_2) or an amino-protecting group;

aa^1 is an amino acid having a hydrocarbyl side chain containing no more than 20 carbon atoms and comprising at least one cyclic group having up to 13 carbon atoms;

aa^2 is an imino acid having from 4 to 6 ring members;

R^1 is a group of the formula $-(CH_2)_s-Z$, where s is 2, 3 or 4 and Z is –OH, –OMe, –OEt or halogen (F, Cl, Br or I).

10. A salt of claim 9 wherein aa^1 is selected from Phe, Dpa and wholly or partially hydrogenated analogues thereof.

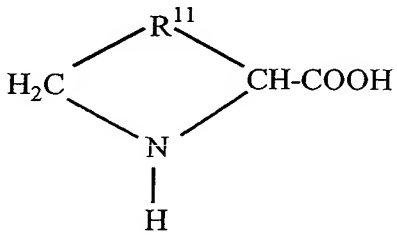
11. A salt of claim 9 wherein aa^1 is selected from Dpa, Phe, Dcha and Cha.

12. A salt of any of claims 9 to 11 wherein aa^1 is of R-configuration.

13. A salt of claim 9 wherein aa^1 is (R)-Phe (that is, D-Phe) or (R)-Dpa (that is, D-Dpa).

14. A salt of claim 9 wherein aa^1 is (R)-Phe.

15. A salt of any of claims 9 to 15 wherein aa^2 is a residue of an imino acid of formula (IV)



(IV),

where R^{11} is $-CH_2-$, $-CH_2-CH_2-$, $-S-CH_2-$, $-S-C(CH_3)_2-$ or $-CH_2-CH_2-CH_2-$, which group, when the ring is 5- or 6- membered, is optionally substituted at one or more $-CH_2-$ groups by from 1 to 3 C_1-C_3 alkyl groups.

16. A salt of claim 15 wherein aa^2 is of S-configuration.

17. A salt of claim 15 wherein aa^2 is a natural proline residue.

18. A salt of claim 9, wherein aa^1-aa^2 is (R)-Phe-(S)-Pro (that is, D-Phe-L-Pro).

19. A salt of any of claims 9 to 18 wherein R^1 is 2-bromoethyl, 2-chloroethyl, 2-methoxyethyl, 3-bromopropyl, 3-chloropropyl or 3-methoxypropyl.

20. A salt of any of claims 9 to 18 wherein R^1 is 3-methoxypropyl.

21. A salt of any of claims 9 to 20 where X is $R^6-(CH_2)_p-C(O)-$, $R^6-(CH_2)_p-S(O)_2-$, $R^6-(CH_2)_p-NH-C(O)-$ or $R^6-(CH_2)_p-O-C(O)-$ wherein p is 0, 1, 2, 3, 4, 5 or 6 and R^6 is H or a 5 to 13-membered cyclic group optionally substituted by 1, 2 or 3 substituents selected from halogen, amino, nitro, hydroxy, a C_5-C_6 cyclic group, C_1-C_4 alkyl and C_1-C_4 alkyl containing, and/or linked to the cyclic group through, an in-chain O, the aforesaid alkyl groups optionally being substituted by a substituent selected from halogen, amino, nitro, hydroxy and a C_5-C_6 cyclic group.

22. A salt of claim 21 wherein said 5 to 13-membered cyclic group is aromatic or heteroaromatic.

23. A salt of claim 22 wherein said 5 to 13-membered cyclic group is phenyl or a 6-membered heteroaromatic group.

24. A salt of any of claims 9 to 20 wherein X is R^6-$(CH_2)_p$-C(O)- or R^6-$(CH_2)_p$-O-C(O)- and p is 0 or 1.

25. A salt of any of claims 9 to 20 wherein X is benzyloxycarbonyl.

26. A salt of claim 9 which is a salt of a compound of formula (VIII):

X-(R)-Phe-(S)-Pro-(R)-Mpg-$B(OH)_2$ (VIII).

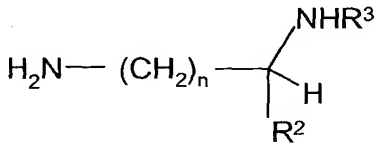
27. A salt of any of claims 1 to 26 which comprises boronate ions derived from the boronic acid and monovalent counter-ions.

28 A salt of any of claims 1 to 26 which comprises a salt of the peptide boronic acid with an alkali metal or a strongly basic organic nitrogen-containing compound.

29. A salt of claim 28 wherein the strongly basic organic nitrogen-containing compound is a guanidine, a guanidine analogue or an amine.

30. A salt of any of claims 1 to 27 which is a salt of the boronic acid with a metal.

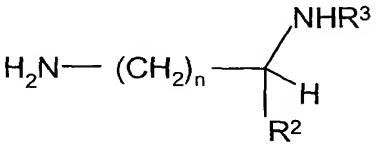
31. A salt of any of claims 1 to 26 which comprises a salt of the boronic acid with an alkali metal, an aminosugar, a guanidine or an amine of formula (XI):



(XI)

where n is from 1 to 6, R^2 is H, carboxylate or derivatised carboxylate, R^3 is H, C_1-C_4 alkyl or a residue of a natural or unnatural amino acid.

32. A salt of any of claims 1 to 26 which comprises a salt of the boronic acid with a guanidine or with an amine of formula (IX):



(IX)

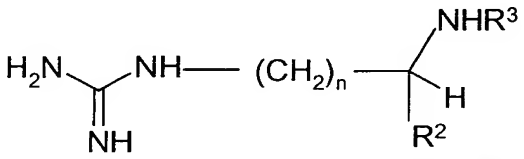
where n is from 1 to 6, R^2 is H, carboxylate or derivatised carboxylate, R^3 is H, C_1-C_4 alkyl or a residue of a natural or unnatural amino acid.

33. A salt of claim 32 which comprises a guanidine salt of the boronic acid.

34. A salt of claim 33 which comprises a salt of the boronic acid with L-arginine or an L-arginine analogue.

35. A salt of claim 34 wherein the L-arginine analogue is D-arginine, or the D- or L- isomers of homoarginine, agmatine [(4-aminobutyl) guanidine], NG-nitro-L-arginine methyl ester, or a 2-amino pyrimidines.

36. A salt of claim 33 which comprises a salt of the boronic acid with a guanidine of formula (VII)



(VII)

where n is from 1 to 6, R^2 is H, carboxylate or derivatised carboxylate, R^3 is H, C_1-C_4 alkyl or a residue of a natural or unnatural amino acid.

37. A salt of claim 36, wherein n is 2, 3 or 4.

38. A salt of claim 36 or claim 37 where the derivatised carboxylate forms a C_1-C_4 alkyl ester or amide.

39. A salt of any of claims 36 to 38 wherein the compound of formula (VII) is of L-configuration.

40. A salt of claim 33 which comprises an L-arginine salt of the peptide boronic acid.

41. A salt of claim 32 which comprises a salt of the boronic acid with an amine of formula (IX).

42. A salt of claim 41, wherein n is 2, 3 or 4.

43. A salt of claim 41 or claim 42 where the derivatised carboxylate forms a C_1-C_4 alkyl ester or amide.

44. A salt of any of claims 41 to 43 wherein the amine of formula (IX) is of L-configuration.

45. A salt of claim 41 which comprises an L-lysine salt of the boronic acid.

46. A salt of any of claims 1 to 26 which comprises an alkali metal salt of the boronic acid.

47. A salt of claim 46 wherein the alkali metal is potassium.

48. A salt of claim 46 wherein the alkali metal is sodium.

49. A salt of claim 46 wherein the alkali metal is lithium.

50. A salt of any of claims 1 to 26 which comprises an aminosugar salt of the boronic acid.

51. A salt of claim 50 wherein the aminosugar is a ring-opened sugar.

52. A salt of claim 51 wherein the aminosugar is a glucamine.

53. A salt of claim 50 wherein the aminosugar is a cyclic aminosugar.

54. A salt of any of claims 50 to 53 wherein the aminosugar is N-unsubstituted.

55. A salt of any of claims 50 to 53 wherein the aminosugar is N-substituted by one or two substituents.

56. A salt of claim 55 wherein the or each substituent is a hydrocarbyl group.

57. A salt of claim 55 wherein the or each substituent is selected from the group consisting of alkyl and aryl moieties.

58. A salt of claim 57 wherein the or each substituent is selected from the group consisting of C_1, C_2, C_3, C_4, C_5, C_6, C_7 and C_8 alkyl groups

59. A salt of any of claims 55 to 58 wherein there is a single N-substituent.

60. A salt of claim 50 wherein the glucamine is N-methyl-D-glucamine.

61. A salt of any of claims 1 to 60 which comprises boronate ions derived from the peptide boronic acid and has a stoichiometry consistent with the boronate ions carrying a single negative charge.

62. A salt of any of claims 1 to 60 wherein the salt consists essentially of acid salt (that is, wherein one B-OH group remains protonated).

63. A salt of any of claims 1 to 62 wherein the salt comprises a boronate ion derived from the peptide boronic acid and a counter-ion and wherein the salt consists essentially of a salt having a single type of counter-ion.

64. A product for use as a pharmaceutical, comprising a salt of any of claims 1 to 63.

65. A pharmaceutical formulation in oral dosage form comprising a salt of any of claims 1 to 63 and a pharmaceutically acceptable diluent, excipient or carrier.

66. A pharmaceutical formulation of claim 65 which is adapted to release the salt in the duodenum.

67. A pharmaceutical formulation of claim 66 which is enterically coated.

68. A method of inhibiting thrombin in the treatment of disease comprising administering to a mammal a therapeutically effective amount of an active agent selected from the group consisting of the salts of any of claims 1 to 63.

69. The use of a salt of any of claims 1 to 63 for the manufacture of a medicament for treating thrombosis.

70. A method of treating venous and/or arterial thrombosis by prophylaxis or therapy, comprising administering to a mammal suffering from, or at risk of suffering from, arterial thrombosis a therapeutically effective amount of a product selected form the salts of any of claims 1 to 63.

71. A method of claim 70 wherein the disease is an acute coronary syndrome.

72. A method of claim 70 wherein the disease is acute myocardial infarction.

73. A method of claim 70 wherein the disease is a venous thromboembolic event, selected from the group consisting of deep vein thrombosis and pulmonary embolism.

74. A method for preventing thrombosis in a haemodialysis circuit of a patient, comprising administering to the patient a therapeutically effective amount of a product selected from the salts of any of claims 1 to 63.

75. A method for preventing a cardiovascular event in a patient with end stage renal disease, comprising administering to the patient a therapeutically effective amount of a product selected from the salts of any of claims 1 to 63.

76. A method for preventing venous thromboembolic events in a patient receiving chemotherapy through an indwelling catheter, comprising administering to the patient a therapeutically effective amount of a product selected from the salts of any of claims 1 to 63.

77. A method for preventing thromboembolic events in a patient undergoing a lower limb arterial reconstructive procedure, comprising administering to the patient a therapeutically effective amount of a product selected from the salts of any of claims 1 to 63.

78. A method of inhibiting platelet procoagulant activity, comprising administering to a mammal at risk of, or suffering from, arterial thrombosis a therapeutically effective amount of a product selected from the salts of any of claims 1 to 63.

79. A method of claim 78 wherein the disease is an acute coronary syndrome.

80. A method of treating by way of therapy or prophylaxis an arterial disease selected from acute coronary syndromes, cerebrovascular thrombosis, peripheral arterial occlusion and arterial thrombosis resulting from atrial fibrillation, valvular heart disease, arterio-venous shunts, indwelling catheters or coronary stents, comprising administering to a mammal a therapeutically effective amount of a product selected from the salts of any of claims 1 to 63.

81. A method of claim 80 wherein the disease is an acute coronary syndrome.

82. The use of a salt of any of claims 1 to 63 for the manufacture of a medicament for a treatment recited in any of claims 76 to 81.

83. A pharmaceutical formulation comprising a combination of (i) a salt of any of claims 1 to 63 and (ii) a further pharmaceutically active agent.

84. A pharmaceutical formulation comprising a combination of (i) a salt of any of claims 1 to 63 and (ii) another cardiovascular treatment agent.

85. A formulation of claim 84 wherein the other cardiovascular treatment agent comprises a lipid-lowering drug, a fibrate, niacin, a statin, a CETP inhibitor, a bile acid sequestrant, an anti-oxidant, a IIb/IIIa antagonist, an aldosterone inhibitor, an A2 antagonist, an A3 agonist, a beta-

blocker, acetylsalicylic acid, a loop diuretic, an ace inhibitor, an antithrombotic agent with a different mechanism of action, an antiplatelet agent, a thromboxane receptor and/or synthetase inhibitor, a fibrinogen receptor antagonist, a prostacyclin mimetic, a phosphodiesterase inhibitor, an ADP-receptor (P_2 T) antagonist, a thrombolytic, a cardioprotectant or a COX-2 inhibitor.

86. The use of a salt of any of claims 1 to 63 for the manufacture of a medicament for treating, for example preventing, a cardiovascular disorder in co-administration with another cardiovascular treatment agent.

87. A method for recovering from ether solution an ester of a boronic acid as defined in any of claims 1 to 26, comprising dissolving diethanolamine in the solution, allowing or causing a precipitate to form and recovering the precipitate.

88. A method of claim 79 wherein the ester is a pinacol ester.

89. The method of claim 79 or claim 80 which further comprises converting the precipitated material into the free organoboronic acid.

90. The method of claim 89, wherein the conversion comprises contacting the precipitated material with an aqueous acid or base.

91. The method of claim 90, wherein the precipitated material is contacted with a concentrated strong inorganic acid.

92. A method for making a boronic acid as defined in any of claims 1 to 26, comprising converting a diolamine reaction product thereof to the acid.

93. The method of claim 92, wherein the conversion is carried out as recited in claim 82 or claim 83.

94. The method of any of claims 87 to 93, which further comprises converting the organoboronic acid to a salt thereof.

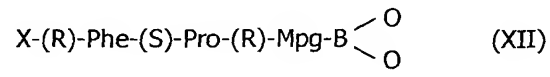
95. The method of claim 94, wherein the salt is as defined in any of claims 2 to 63.

96. The method of claim 94 or claim 95, which further comprises formulating the salt into a pharmaceutical composition.

97. A product obtainable by (having the characteristics of a product obtained by) reacting in diethylether solution a pinacol ester of a compound of Formula (VIII) as defined in claim 26 and diethanolamine.

98. A composition of matter comprising:

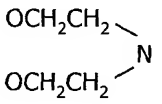
(i) a species of formula (XII)



(XII)

wherein X is H or an amino protecting group, the boron atom is optionally coordinated additionally with a nitrogen atom, and the valency status of the terminal oxygens is open (they may be attached to a second covalent bond, be ionised as $-O^-$, or have some other, for example intermediate, status); and, in bonding association therewith

(ii) a species of formula (XIII)



(XIII)

wherein the valency status of the nitrogen atom and the two oxygen atoms is open.

99. A composition of claim 98, wherein the terminal oxygen atoms of the species of formula (XII) and the oxygen atoms of the species of formula (XIII) are the same oxygen atoms, i.e. the species of formula (XIII) forms a diol ester with the species of formula (XII).

100. The use of a boronic acid as defined in any of claims 1 to 26 as an intermediate to make a salt of any of claims 1 to 63.

101. A method of preparing a salt of any of claims 1 to 63, comprising contacting a boronic acid as defined in any of claims 1 to 26 with a base capable of making such a salt.

102. A peptide boronic acid of formula (II) as defined in any of claims 9 to 26 when of GLP or GMP quality, or when in compliance with GLP (good laboratory practice) or GMP (good manufacturing practice).

103. A composition of matter which is sterile or acceptable for pharmaceutical use, or both, and comprises a peptide boronic acid of formula (II) as defined in any of claims 9 to 26.

104. A composition of matter of claim 103 which is in particulate form.

105. A composition of claim 103 which is in the form of a liquid solution or dispersion.

106. An isolated compound which is a peptide boronic acid of formula (VIII):

X-(R)-Phe-(S)-Pro-(R)-Mpg-$B(OH)_2$ (VIII)

wherein X is H (to form NH_2) or an amino-protecting group.

107. A compound of claim 106 wherein X is benzyloxycarbonyl.

108. A particulate composition comprising a peptide boronic acid of formula (VIII) as defined in claim 106 or claim 107.

109. A composition of claim 108 consisting predominantly of the peptide boronic acid.

110. A composition of claim 109 wherein the peptide boronic acid forms at least 75% by weight of the composition.

111. A composition of claim 110 wherein the peptide boronic acid forms at least 85% by weight of the composition.

112. A composition of claim 111 wherein the peptide boronic acid forms at least 95% by weight of the composition.

113. A composition of any of claims 108 to 112 which is sterile.

114. A composition of any of claims 108 to 113 wherein the peptide boronic acid is in finely divided form.

115. A liquid composition consisting of, or consisting essentially of, a peptide boronic acid of formula (II) as defined in any of claims 9 to 26 and liquid vehicle in which it is dissolved or suspended.

116. A liquid composition of claim 115 wherein the liquid vehicle is an aqueous medium, e.g. water.

117. A liquid composition of claim 115 wherein the liquid vehicle is an alcohol, for example methanol, ethanol, isopropanol or another propanol, another alkanol or a mixture of the aforegoing.

118. A liquid composition of any of claims 115 to 117 which is sterile.

119. A medicament comprising a salt of a boronic acid which is a selective thrombin inhibitor and has a neutral aminoboronic acid residue capable of binding to the thrombin S1 subsite linked through a peptide linkage to a hydrophobic moiety capable of binding to the thrombin S2 and S3 subsites, the salt comprising a cation having a valency V and having an observed stoichiometry consistent with a notional stoichiometry (boronic acid:cation) of V:1.

120. A medicament of claim 119 wherein the boronic acid has a Ki for thrombin of about 100 nM or less.

121. A medicament of claim 119 wherein the boronic acid has a Ki for thrombin of about 20 nM or less.

122. A medicament comprising a sodium salt of Cbz-(R)-Phe-(S)-Pro-(R)-Mpg-$B(OH)_2$.

123. A method of stabilising an organoboronic acid, comprising providing it in the form of a salt thereof.

124. A method of formulating an organoboronic acid drug to increase the stability of the drug species, comprising formulating the acid in the form of an acid salt thereof.

ABSTRACT OF THE DISCLOSURE

BOROPEPTIDES

Salts of a peptide boronic acid drug, for example of Cbz-(R)-Phe-(S)-Pro-(R)-Mpg-$B(OH)_2$. The counter-ion to the boronate may be an alkali metal or derived from a strongly basic organic nitrogen-containing compound.

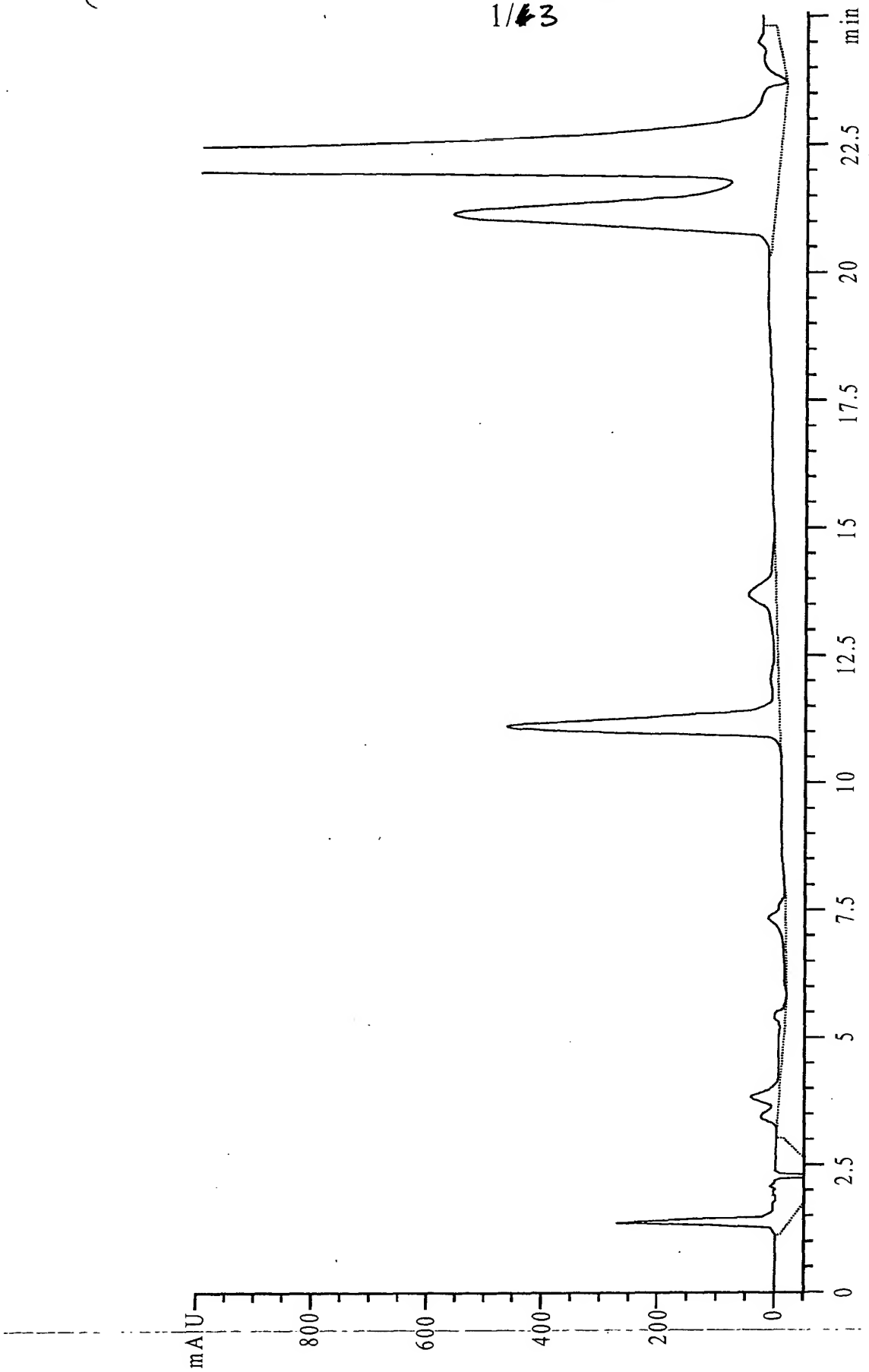


Fig. 1

2/3

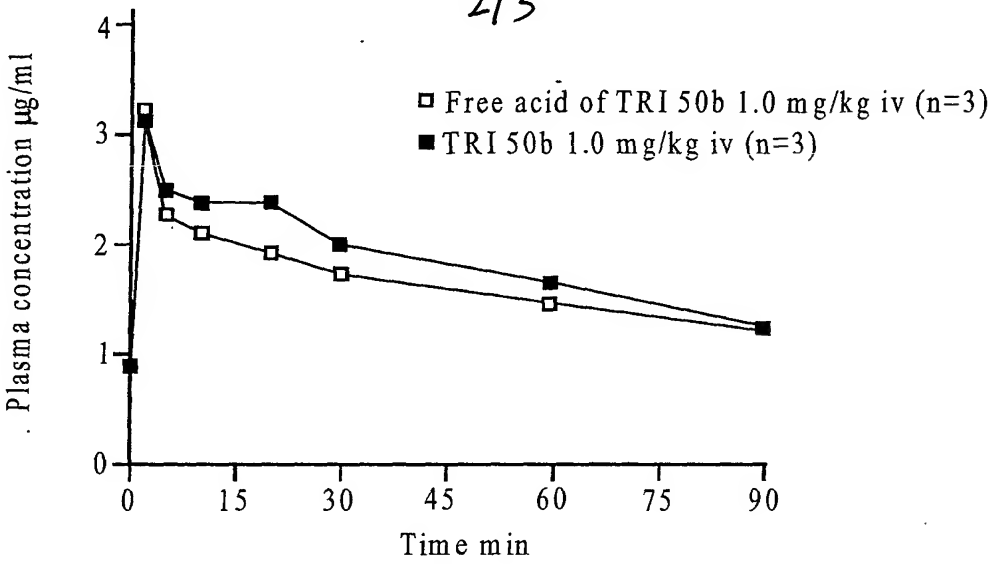

Plasma concentration µg/ml
0
1
2
3
4
0
15
30
45
60
75
90
Time min
Free acid of TRI 50b 1.0 mg/kg iv (n=3)
TRI 50b 1.0 mg/kg iv (n=3)

Fig. 2

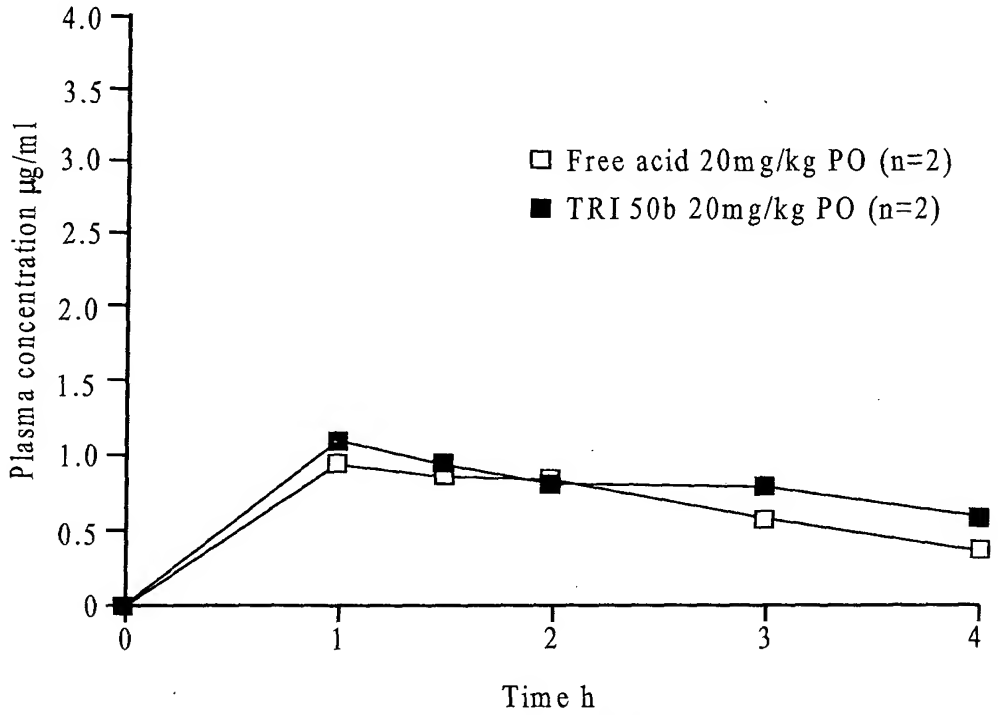

Plasma concentration µg/ml
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
0
1
2
3
4
Time h
Free acid 20mg/kg PO (n=2)
TRI 50b 20mg/kg PO (n=2)

Fig. 3

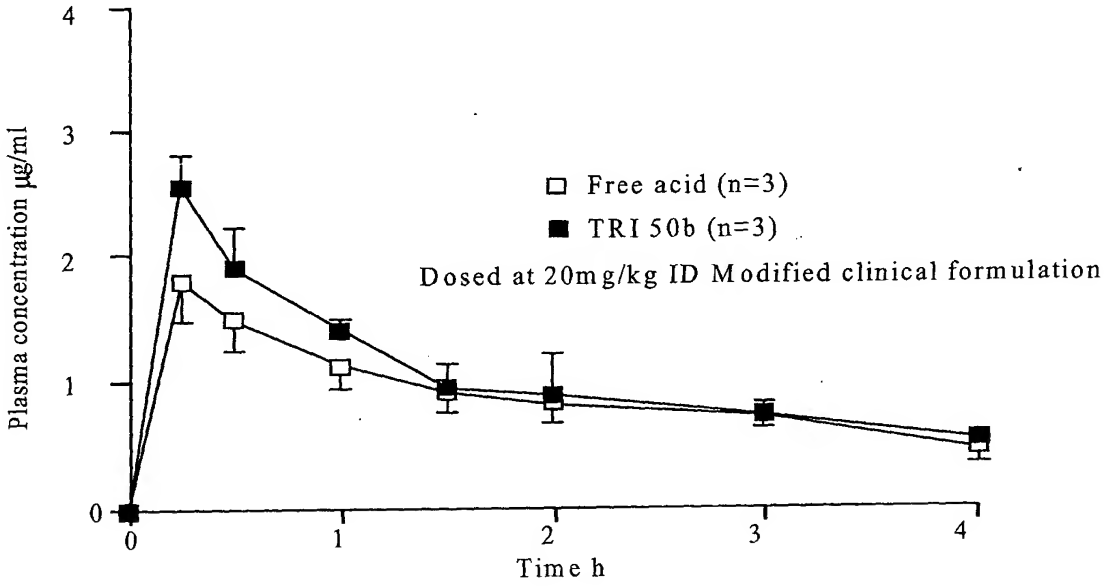

Plasma concentration µg/ml
4
3
2
1
0
0
1
2
3
4
Time h
Free acid (n=3)
TRI 50b (n=3)
Dosed at 20mg/kg ID Modified clinical formulation

Fig. 4